JAN 8 1945

CANCER RESEARCH

Medical Library

VOLUME 5
NUMBER 1
JANUARY, 1945

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

CONTENTS

W. L. Simpson, C. Carruthers, and W. Cramer. Loss of Carcinogenic Activity when Methylcholanthrene is Dissolved in Anhydrous Lanolin 1

W. L. Simpson, and W. Cramer. Sensitization of Skin by Carcinogenically Inactive Methylcholanthrene to Subsequent Carcinogenesis 5

F. Duran-Reynals, E. W. Shrigley, and E. de Hostos. Carcinogenesis Induced by Methylcholanthrene in Pigeons, Guinea Fowls, and Ducks 11

Van R. Potter, and Gertrude J. Liebl. Biocatalysts in Cancer Tissue. V. Adenosinetriphosphatase 18

Van R. Potter, W. C. Schneider, and Gertrude J. Liebl. Enzyme Changes During Growth and Differentiation in the Tissues of the Newborn Rat 21

F. Duran-Reynals. Immunological Factors that Influence the Neoplastic Effects of the Rabbit Fibroma Virus..... 25

Robert A. Huseby, Zelda B. Ball, and Maurice B. Visscher. Further Observations on the Influence of Simple Caloric Restriction on Mammary Cancer Incidence and Related Phenomena in C3H Mice. 40

Edward W. Wallace, Helene Wallace, and C. A. Mills. Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in Spayed C3H Mice..... 47

F. L. Warren. Estimation of Urinary 17-Ketosteroids in the Diagnosis of Adrenal Cortical Tumors..... 49

The American Association for Cancer Research, Inc. Special Meeting of the Board of Directors, October 12, 1944..... 55

Abstracts 56-61
Experimental Research, Animal Tumors..... 56-59
Clinical and Pathological Reports..... 59-61

Book Reviews 62-64

THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

This journal is sponsored by the American Association for Cancer Research, Inc., The Anna Fuller Fund, The International Cancer Research Foundation, and The Jane Coffin Childs Memorial Fund for Medical Research.

Advisory Board

MILDRED W. S. SCHRAM, *Chairman*

S. BAYNE-JONES — JAMES B. MURPHY

C. C. LITTLE — GEORGE M. SMITH

Editorial Committee

JAMES B. MURPHY, *Chairman* — WM. H. WOGLOM, *Secretary*

CLARA J. LYNCH, *Editor, Abstracts Section*

JOHN J. BITTNER
ALEXANDER BRUNSCHWIG
E. V. COWDRY
LOUIS I. DUBLIN
GIOACCHINO FAILLA
LOUIS F. FIESER
JACOB FURTH
WILLIAM U. GARDNER
JESSE P. GREENSTEIN
FRANCES L. HAVEN
BALDUIN LUCKÉ
E. C. MACDOWELL
G. BURROUGHS MIDER
EDGAR G. MILLER
JOHN J. MORTON
EDITH H. QUIMBY
MURRAY J. SHEAR
HAROLD L. STEWART
GRAY H. TWOMBLY
SHIELDS WARREN

Abstractors

W. A. BARNES
S. BAYNE-JONES
M. BELKIN
E. BOYLAND
J. B. BRIGGS
R. BRIGGS
W. J. BURDETTE
A. CLAUDE
P. P. COHEN
A. CORNELL
H. G. CRABTREE
H. J. CREECH
Z. DISCHE
C. E. DUNLAP
T. B. DUNN
M. DURAN-REYNALS
W. E. GYE
A. HADDOW
J. B. HAMILTON
F. L. HAVEN
I. HIEGER
H. HOGEBOOM
M. E. HOWARD
R. N. JONES
E. L. KENNAWAY
J. G. KIDD
A. KIRSCHBAUM
E. A. LAWRENCE
R. J. LUDFORD
V. F. MARSHALL
W. V. MAYNEORD
J. L. MELNICK
C. A. PFEIFFER
K. R. PORTER
L. W. PRICE
E. H. QUIMBY
E. C. RICHARDSON
D. SHEMIN
R. E. SNYDER
E. E. SPROUL
K. G. STERN
C. WARREN
F. L. WARREN
H. Q. WOODARD
G. W. WOOLLEY

Published by The International Cancer Research Foundation.

Publication Office, 1500 Greenmount Ave., Baltimore 2, Maryland.

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Robert W. Briggs, Business Manager, 1500 Greenmount Ave., Baltimore 2, Md., or 1916 Lincoln-Liberty Building, Philadelphia 7, Pa.

No responsibility is accepted by the Committee, by the Board, or by the Publishers of *Cancer Research* for opinions expressed by contributors.

Entered as second class matter February 12, 1941, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

Copyright, 1945, by The International Cancer Research Foundation.

SEE INSIDE BACK COVER FOR INFORMATION FOR CONTRIBUTORS

CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 5 JANUARY, 1945 NUMBER 1

Loss of Carcinogenic Activity when Methylcholanthrene is Dissolved in Anhydrous Lanolin*

W. L. Simpson, Ph.D., C. Carruthers, Ph.D., and W. Cramer, Ph.D., M.R.C.S.

(From the Research Department of The Barnard Free Skin and Cancer Hospital, and the Department of Anatomy of Washington University School of Medicine, St. Louis 3, Missouri)

(Received for publication August 14, 1944)

As reported in a previous paper (9) microscopic examination in ultraviolet light of sections of mouse skin to which a benzene solution of methylcholanthrene had been applied revealed the fact that a large fraction of the carcinogen had passed into the sebaceous glands, where it became dissolved in the sebum. The glands subsequently degenerated and the sebum with the methylcholanthrene dissolved in it was expelled and spread over the surface of the skin. These findings raised the question how the carcinogenic action of methylcholanthrene was affected when the carcinogen was dissolved in sebum. In order to investigate this point methylcholanthrene was dissolved in anhydrous lanolin—the sebum of sheep skin. It was found (10) that the lanolin solution of the carcinogen failed to produce the carcinogenic effect of methylcholanthrene when applied to the skin of mice. It did not even induce any of the other preliminary changes that follow the application of a benzene solution or an acetone solution of this carcinogen, such as epilation, destruction of the sebaceous glands, or a nonneoplastic epithelial hyperplasia.

In order to obtain further information on the nature of this surprising phenomenon 3 additional experimental series have been carried out in which methylcholanthrene dissolved in anhydrous lanolin was applied to the skin of mice. This paper gives a detailed account of these later experiments and includes also the results of the first experiment, which have already been published. The experiments are designated in this paper by the letters A, B, C, and D; A being the previously reported experiment.

METHODS

The technic was the same in all 4 series, and young female mice of the Swiss strain were used in all. For experiments A and B, the animals were supplied by Albino Farms, for experiments C and D, by Tumblebrook Farm, which had been our usual source of supply for this strain. Methylcholanthrene was dissolved in melted anhydrous lanolin to obtain a 0.3 per cent solution of the carcinogen by weight. This concentration was chosen in order to ensure the application to the skin of amounts of the carcinogen that would be comparable to the amounts applied in our previous experiments with a benzene solution carried out for the purpose of standardizing the carcinogenic response (4-6). In those experiments 0.3, 0.6, and 1 per cent solutions of methylcholanthrene were applied by one brush stroke to a large area of the skin of the back 3 times weekly for 14 weeks (42 applications). With a benzene solution of methylcholanthrene it had been found that an increase in the concentration from 0.3 to 1 per cent was of relatively slight importance in determining the carcinogenic response, if the latter is expressed in terms of cancerous animals. After 16 weeks the results were 60 per cent of cancerous mice for the 1 per cent solution, 45 per cent for the 0.6 per cent solution and 40 per cent for the 0.3 per cent solution, while papillomas were present at that time in about 30 per cent of the animals. After 26 weeks the carcinogenic response rose to 90 per cent for the lowest and to 100 per cent for the highest concentration.

The anhydrous lanolin solution has to be melted in a warm water bath before application with a brush and, owing to its viscous nature, a much larger amount of it is applied than when a benzene solution is used; in fact, more than twice as much. For comparison with the 0.6 per cent concentration of methylcholanthrene in benzene used in most of our previous experiments, a concentration of 0.3 per cent of the carcinogen in anhydrous lanolin was therefore considered to ensure the application of comparable amounts of the carcinogen.

* Aided by grants from an anonymous donor and from The International Cancer Research Foundation.

As in our experiments with the benzene solution of the carcinogen, 42 applications of the lanolin solution were given by a single brush stroke 3 times weekly to a large area of the skin of the back.

RESULTS

The results of the 4 experiments are summarized in Table I. The carcinogenic response given there refers to the conditions observed 16 weeks after the first application. In experiment A, in which 1 mouse out of 50 had developed a papilloma, all the animals were kept under observation for 12 weeks after the last application without subjecting them to any further experimental test. At the end of this period the 49 animals that were negative in the 16th week still remained negative. The 1 exceptional mouse that had developed a papilloma at the 16th week died in the 23rd week of the experiment. On microscopic examination the papilloma was found to be of a precancerous nature, but not yet malignant. Since this animal would probably have developed a carcinoma by the end of the experiment if it had survived, the total carcinogenic response after 6 months in this experiment was taken to be 2 per cent if calculated in terms of cancerous animals. After 16 weeks there had been no carcinogenic response. Since in experiment A all the mice negative at the 16th week had remained negative during the following 10 weeks, it was justifiable to assume that the mice that failed to show any macroscopically visible change, such as epilation or ulceration, at the end of the 16th week in experiments B, C, and D would continue to remain negative, and could be used for testing the biological response of the skin to a benzene solution of methylcholanthrene. The results of such tests will be described in the following paper. In any case the carcinogenic response, as measured at the 16th week, gives an accurate measure of comparison with the response obtained during the same period with a benzene solution of the carcinogen. Such a comparison shows that in the 16th week only 3 of 312 mice showed pathological changes; namely, 1 papilloma in experiment A and 1 carcinoma and 1 nonmalignant ulceration in experiment C. The 2 animals with noncancerous lesions at the 16th week subsequently developed carcinomas at the sites of these lesions. We know from previous experiments with this strain that methylcholanthrene in benzene, applied 3 times weekly for 14 weeks to 312 mice, would have elicited in 16 weeks fully developed carcinomas in more than 100 animals and papillomas or precancerous ulcerations in a similar number, while only about 100 mice would have remained negative. At the end of 26 weeks each of the surviving mice that had been treated with the benzene solution of the carcinogen would have developed at least 1 carcinoma, and some of them 2, 3, or 4 carcinomas.

TABLE I

Experiment	Serial number	Source of Swiss strain	Number of mice: Effective	Number of mice: With pathological changes after 16 weeks	Details of pathological changes	Subsequent history
A	LXVIII	Albino Farms	50	1	Small papilloma	Mouse died 23 weeks after 1st application with a precancerous papilloma
B	LXXVI	" "	60	0	—	—
C	XCV	Tumblebrook Farm	99	2	1 carcinoma after 14 weeks	
					1 area of epilation with small shallow ulceration	Carcinoma 42 weeks after 1st painting
D	VII	" "	103	0	—	—
Total number of mice			312		Total number of mice with carcinoma	3

Sections of the skin examined from a number of animals of experiments B, C, and D showed the same normal appearance that had been observed in the skin of mice from experiment A, as illustrated in our previous paper by photomicrographs (10).

In a systematic investigation of the carcinogenic process carried out by the Barnard Hospital group, Carruthers and Suntzeff (3, 11) have examined the changes in some of the mineral constituents of mouse epidermis that result from the application of methylcholanthrene in benzene. They found that only two of them, calcium and iron, showed a very definite change; namely, a pronounced fall. A single application of methylcholanthrene in benzene was sufficient to reduce the epidermal content of these cations within 10 days to approximately 50 per cent of the normal. Repeated applications continued over periods of 30 or even 60 days produced a further reduction of the calcium content. Similar results were obtained for iron.

Determinations of iron and calcium content were made on the skin of mice that had received 42 applications of a lanolin solution of methylcholanthrene in 14 weeks. These estimations were carried out by the technic used for the analysis of skin treated with a solution of methylcholanthrene in benzene. The epidermis was analyzed after it had been separated from the dermis by heat and blunt dissection (2). For each individual analysis the separated epidermis from 10 animals was used. The results are given in Table II, together with the values for normal skin and for skin after treatment with a benzene solution of methylcholanthrene, as recorded in the papers by Carruthers and Suntzeff. The results show that the suppression of the carcinogenic activity of methylcholanthrene, when this compound is dissolved in anhydrous lanolin, has also suppressed its power to reduce the calcium and iron content of the epidermis. This result lends strong support to the contention that the reduction of the calcium and iron content of the epidermis represents an essential step in the initiation of the carcinogenic process in the skin.

TABLE II

Mice used for analysis	Number of paintings	Interval from first painting to killing, days	Fe micrograms per 100 mgm. epidermis	Ca micrograms per 100 mgm. epidermis
Normal controls*	0		4.6 to 7.7 average 6.4	36 to 52 average 43.5
Painted with 0.3% methylcholanthrene in anhydrous lanolin.	37 to 42	100 to 120	4.8 6.4 7.4 average 6.2	47 49
Painted with 0.6% methylcholanthrene in benzene.*	1 2 3 6 to 9 13 " 33	10 10 10 15 to 26 30 " 70	3.3 3.2 2.4 2.5 1.6	22 19 16 14 to 22

* Data from earlier papers by Carruthers and Suntzeff (3, 11).

Another striking effect of methylcholanthrene that has recently been investigated at the Barnard Hospital is its capacity to produce an increase in the volume of the chromosomes of many epidermal cells when applied in solution in benzene (1). Skins treated with a lanolin solution of the carcinogen might be expected to throw some light on whether these chromosomal alterations are associated with the carcinogenic response by the skin or merely represent a side effect of methylcholanthrene. Such studies have been undertaken and will be reported separately.

The absorption by the skin of methylcholanthrene dissolved in anhydrous lanolin was studied in ultraviolet light after a single application and after prolonged treatment with this solution. The fluorescence microscopic methods were the same as those employed in our earlier work with benzene solutions of the carcinogen (9). Since anhydrous lanolin is a rather viscous solvent, it was not surprising to find that much less fluorescent methylcholanthrene was present in the sebaceous glands than is visible after a single application of the benzene solution. The glands did, however, contain enough of the carcinogen to emit a distinct blue-violet fluorescence, but it was much less intense than that of the film of dissolved carcinogen coating the external surface of the skin. No methylcholanthrene fluorescence was detectable in the fat cells of the dermis or subdermal layers at any time. Within 24 to 36 hours the last traces of methylcholanthrene fluorescence were gone from the skin. Sections examined at these intervals appeared like normal untreated skin, and confirmed the impression obtained from macroscopic examination of the mice in filtered ultraviolet light, that the carcinogen had completely disappeared. This can probably be accounted for partially by the rubbing off of the greasy film against the cage and the shavings in it. The balance must have been absorbed, and either excreted or destroyed with such rapidity that no local accumulations were detectable in any part of the skin after a day or so.

The skins of several mice that had been subjected to a frequently repeated treatment with an anhydrous lanolin solution of methylcholanthrene were examined at intervals from 1 to 13 weeks after the last treatment with the lanolin solution. In no sample of skin was there found any abnormal fluorescence to suggest the accumulation or persistence of methylcholanthrene or its fluorescent derivatives.

DISCUSSION

While the inhibition by lanolin of carcinogenesis proper, *i.e.*, the production of carcinomas, has not been studied previously, there is a considerable body of experimental evidence on the inhibition of the biological activity of chemical carcinogens by the fatty media in which they are dissolved, when the test

is the development of sarcomas in response to subcutaneous injections. Weil-Malherbe and Dickens (7, 12), who give a survey of previous work, confirmed the findings of Peacock and Beck, and of Morton and Mider that the body fat of the mouse, homologous fat, inhibited sarcogenesis. But they also found that the lipids of ox brain, a nonhomologous fat, had the same inhibiting effect. In a recent paper the effects of two purified constituents of ox brain were studied. Both lecithin and cephalin inhibited sarcogenesis. In contrast to this inhibiting effect, cod liver oil, which is rich in unsaturated fatty acids, had no inhibitory effect, but actually favored the development of sarcomas.

Leiter and Shear (8) studied the effect of lard as a vehicle for carcinogens injected subcutaneously. Using different fractions of lard they found great variations in their effect on the sarcogenic process, only some fractions showing a pronounced retardation. After an extensive investigation they arrived at the conclusion that the retarding influence resided in the glycerides richest in saturated fatty acids of high molecular weight.

This work on the inhibition of sarcogenesis shows that the inhibitory effect is a property shared by many different fatty materials and is, therefore, likely to be exhibited also by lanolin. Considering all the evidence together, it is probable that an inhibition of the biological effects of chemical carcinogens by fatty materials is not restricted to the epithelial cells of the skin, and may apply also to the epithelial cells of other organs.

The inhibition of carcinogenesis proper in the skin by lanolin has a special significance in so far as it reproduces in the form of a model experiment the conditions actually obtaining in the development of skin cancer in man, as seen in some occupational cancers. The skin is normally covered by sebum, a material closely resembling lanolin, and the immediate effect of applying a chemical carcinogen to its surface is that the compound becomes dissolved in the sebum, as demonstrable by microscopic examination in ultraviolet light. If the carcinogen has been applied dissolved in a lipid solvent such as benzene, there follows a destruction of the sebaceous glands, a change that does not take place if the lipid solvent is omitted. The destruction of the sebaceous glands is accompanied by other morphological and chemical changes in the epidermis that represent the initiation of the carcinogenic process.

SUMMARY AND CONCLUSION

Experiments on 312 mice have shown that methylcholanthrene when dissolved in anhydrous lanolin, a vehicle closely resembling sebum, and applied to the skin of mice, loses its carcinogenic effect. After 42 applications, given 3 times weekly for 14 weeks, it fails to induce skin cancer. It does not even induce those early changes that represent the initiation of the carcinogenic process, such as epilation, destruction of the sebaceous glands, epidermal hyperplasia, and a diminution in the calcium and iron content of the epidermis, which follow the single application of a benzene or acetone solution of the carcinogen. This loss of carcinogenic activity cannot be attributed to a lack of absorption of the carcinogen by the skin. After 42 applications of the lanolin solution of methylcholanthrene to the skin the morphological and chemical criteria that we have studied reveal no differences from normal skin. There is, however, a biological change in the skin, in that it has become sensitized to the action of a benzene solution of methylcholanthrene. This effect will be described in the following paper.

REFERENCES

1. Biesele, J. J., and Cowdry, E. V. Chromosomal Changes in Epidermal Carcinogenesis. J. Nat. Cancer Inst., **4**: 373-384. 1944.
2. Baumberger, J. P., Suntzeff, V., and Cowdry, E. V. Methods for the Separation of Epidermis from Dermis and Some Physiologic and Chemical Properties of Isolated Epidermis. J. Nat. Cancer Inst., **2**:413-423. 1942.
3. Carruthers, C., and Suntzeff, V. Influence of Limited Application of Methylcholanthrene upon Epidermal Iron and Ascorbic Acid. J. Nat. Cancer Inst., **3**:217-220. 1942.
4. Cramer, W., and Stowell, R. E. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. J. Nat. Cancer Inst., **2**:369-378. 1942.
5. Cramer, W., and Stowell, R. E. The Effects of Solvents in Methylcholanthrene Epidermal Carcinogenesis. Cancer Research, **2**:193-197. 1942.
6. Cramer, W., and Stowell, R. E. On the Quantitative Evaluation of Experimental Skin Carcinogenesis by Methylcholanthrene. The Factors of Dosage, Time, Spacing of Applications, and the Multiplicity of the Carcinogenic Response. Cancer Research, **3**:668-681. 1943.
7. Dickens, F., and Weil-Malherbe, H. Factors Affecting Carcinogenesis. I. The Effect of Lipoid Solvents on Tumor Production by 3:4-Benzpyrene. Cancer Research, **2**:560-566. 1942.
8. Leiter, J., and Shear, M. J. Quantitative Experiments on the Production of Subcutaneous Tumors in Strain A Mice with Marginal Doses of 3,4-Benzpyrene. J. Nat. Cancer Inst., **3**:455-477. 1943.
9. Simpson, W. L., and Cramer, W. Fluorescence Studies of Carcinogens in Skin. I. Histological Localization of 20-Methylcholanthrene in Mouse Skin after a Single Application. Cancer Research, **3**:362-369. 1943.
10. Simpson, W. L., and Cramer, W. Sebaceous Glands and Experimental Skin Carcinogenesis in Mice. Cancer Research, **3**:515-518. 1943.
11. Suntzeff, V., and Carruthers, C. The Effect of Methylcholanthrene upon Epidermal Sodium and Calcium. Cancer Research, **3**:431-433. 1943.
12. Weil-Malherbe, H., and Dickens, F. Factors Affecting Carcinogenesis. II. Incorporation of 3,4-Benzpyrene in Media Containing Purified Lecithin or Cephalin. Cancer Research, **4**:425-429. 1944.

Sensitization of Skin by Carcinogenically Inactive Methylcholanthrene to Subsequent Carcinogenesis*

W. L. Simpson, Ph.D., and W. Cramer, Ph.D., M.R.C.S.

(From the Research Department of The Barnard Free Skin and Cancer Hospital, and the Department of Anatomy, Washington University School of Medicine, St. Louis 3, Missouri)

(Received for publication August 14, 1944)

The fact described in the preceding paper (9), that methylcholanthrene dissolved in anhydrous lanolin fails to produce a carcinogenic effect and does not even induce early skin changes such as epilation, epidermal hyperplasia, destruction of sebaceous glands, and diminution in the calcium and iron content of the epidermis, has been established in 4 experimental series comprising 312 mice. Since examination in ultraviolet light demonstrated that the skin absorbs methylcholanthrene from its solution in lanolin, the absence of the carcinogenic effect cannot be attributed to inadequate absorption. Two other possible explanations suggest themselves to account for this phenomenon. One is that the carcinogen exists in lanolin in a physically or chemically inactive state, and the second, that the skin has undergone a biological alteration in the sense of having become resistant to the carcinogen.

In order to investigate this question two experiments have been carried out in which the skin of mice, after having been subjected to prolonged treatment with a lanolin solution of methylcholanthrene, was tested subsequently by applying to it a benzene solution of the carcinogen. The results form the subject matter of this paper. Details of the preliminary treatment with the lanolin solution of methylcholanthrene have already been described (9). The experiments designated here as "A" and "C," which supplied the animals for the subsequent treatment with the benzene solution of the carcinogen, are identical with experiments "A" and "C" of our previous paper, so far as the preliminary treatment with methylcholanthrene in lanolin is concerned.

The two experiments were carried out in essentially the same manner. Young female Swiss mice from 2 to 3 months old were subjected to 42 applications of a 0.3 per cent solution of methylcholanthrene in anhydrous lanolin. These applications were given 3 times weekly for a period of 14 weeks. The animals, which, as a result of this treatment, showed no skin changes and were in good condition, as shown by their increase in weight, were then given 5 applications of a 0.6 per cent benzene solution of methylcholanthrene by means of a single brush stroke. The applications were made at intervals of 3 weeks, so that the last application was given 12 weeks after the first one.

With normal mice this discontinuous technic of administering small total doses of the carcinogen dissolved in benzene had been found by Cramer and Stowell (3, 4) to induce skin cancer in from 40 to 50 per cent of the animals during an interval of 9 months after the first application. As pointed out by these authors, such a technic is better suited to reveal any increase or decrease in the sensitivity of the skin to a carcinogen than is the routine method of applying the carcinogen 3 times weekly for a period of 14 weeks, which results in inducing cancer in 100 per cent of the animals.

The two experiments designed to test the effect of a preliminary treatment with a lanolin solution of methylcholanthrene on the sensitivity of the skin to a benzene solution of that carcinogen differed in only one point. In one, experiment C, the benzene solution of methylcholanthrene was applied for the first time 2 weeks after the last application of the lanolin solution of the carcinogen had been given; in the other, experiment A, an interval of 3 months was allowed to elapse between the last application of the lanolin solution and the first application of the benzene solution. Both experiments were terminated 9 months after the first application of the benzene solution of methylcholanthrene.

The two experimental series, if timed from the first application of the lanolin solution, extended, therefore, over 12 months and 15 months respectively. This is a relatively long period of time, taking into consideration that the maximal life span of the mouse is about 2 years. Since there are on record observations indicating that the response of mouse skin to carcinogens

* Aided by a grant from an anonymous donor.

diminishes with advancing age, it was necessary to use for the control experiments, in which the carcinogen was applied to mice not previously treated with a lanolin solution of methylcholanthrene, animals of the same age as those studied in the two main experimental series. This condition was fulfilled by setting aside, at the beginning of the treatment with the lanolin solution, a reserve of mice of the same age as those subjected to this treatment. The mice used for the controls were taken from this reserve and were subjected to the application of the benzene solution of the carcinogen at the same time, and therefore at the same age, as the group that had received the preliminary treatment with the lanolin solution.

The numbers of effective and of cancerous mice in the two experimental series are given in the following table:

Mice	Experiment A	Control AC	Experiment C	Control CC
Effective	25	34	50	25
Cancerous	23	16	41	12
Percentage with cancer	92	47	82	48

From the time of the first application of the benzene solution of methylcholanthrene, the mice were examined regularly and any skin changes that had occurred were noted in the protocols. In this way the development of papillomas and the subsequent malignant change was determined. Eventually all the painted skin areas were examined histologically, including those that were apparently negative to the naked eye at the end of the experiment.

Fig. 1 shows the development of skin cancer in the two experiments and in the controls. The abscissa gives the time in weeks dated from the first application of the benzene solution of the carcinogen, the ordinate gives the number of mice bearing skin carcinomas expressed in percentage of effective mice. The 5 arrows indicate the applications of methylcholanthrene in benzene. The two control series, AC and CC, show carcinogenic responses that are very similar to each other and very similar, also, to that obtained in previous experiments by Cramer and Stowell and described by them as "incomplete carcinogenesis with discontinuous exposure." The two series, A and C, in which the mice had been subjected to a preliminary course of painting with a lanolin solution of methylcholanthrene, are also very similar to each other, but show a striking contrast with the control series. In these two series the carcinomas develop much earlier and the number of cancerous animals increases much more rapidly than in the control experiments, so that the cancer incidence at the end of the experiment is about double that of the controls. In experiment A no less than 30 per cent of the sensitized mice and only 4 per cent of the controls (*i.e.,* 1 mouse) had fully developed carcinomas at the end of the 12th week, the time at which the last of the 5 applications of methylcholanthrene in benzene was given; in experiment C the figure at the same period is 10 per cent for the sensitized mice and 0 per cent for the controls. A response as early as that of the sensitized mice has never been observed previously in our experiments with the protracted method of application. Very impressive, also, was the contrast between the rapidity with which the appearance of a papilloma was followed by the development of a carcinoma in the sensitized mice and the very prolonged lapse of time required for this process in the control animals.

Although in experiment A an interval of 3 months had elapsed between the preliminary treatment with the lanolin solution and the subsequent treatment with the benzene solution, the carcinogenic response was not less than in experiment C, indicating that the sensitization by the lanolin solution was not a transient effect. Actually the carcinogenic response was somewhat higher in A than in C, but further experiments are necessary to determine whether this can be interpreted as indicating an increased sensitivity or whether this difference lies within the range of experimental variations.

The concept of "cocarcinogens" has been introduced by Shear to designate substances that, though noncarcinogenic when applied alone, are able to enhance the effect of chemical carcinogens when applied together with them. Croton oil and croton resin, for instance, are cocarcinogens, while xylene and turpentine, though irritants, have no cocarcinogenic effects (1). Since in our experiments a potent carcinogen fails to be carcinogenic in the absence of solvents such as benzene, acetone, or chloroform, generally used in the application of carcinogens to the skin, the question had to be considered whether these vehicles might act not merely as solvents but also as cocarcinogens. In order to test this point, mice were submitted to a preliminary painting with a lanolin solution of methylcholanthrene 3 times weekly for 14 weeks. Four weeks were then allowed to elapse in order to ensure the complete disappearance of methylcholanthrene from the skin. Reagent grade benzene was then applied 3 times a week for 18 weeks to the skin of the back of 27 mice that had been thus sensitized. All these mice remained negative, except one that developed a small papilloma. The papilloma persisted as such and remained unchanged till the end of the experiment at the 36th week after the first application of the lanolin solution of methylcholanthrene. The mouse was then killed for microscopic examination

of the tumor, which was found to be a small benign papilloma without any indication of precancerous change. It follows that benzene, which does not induce skin cancer in normal mice, is also ineffective in sensitized mice.

genic response of these treated skins to benzene solutions of methylcholanthrene shows. By elimination, then, we are forced to conclude that the explanation of the lack of carcinogenic effect of the lanolin solution is to be found in the state of the methylcholan-

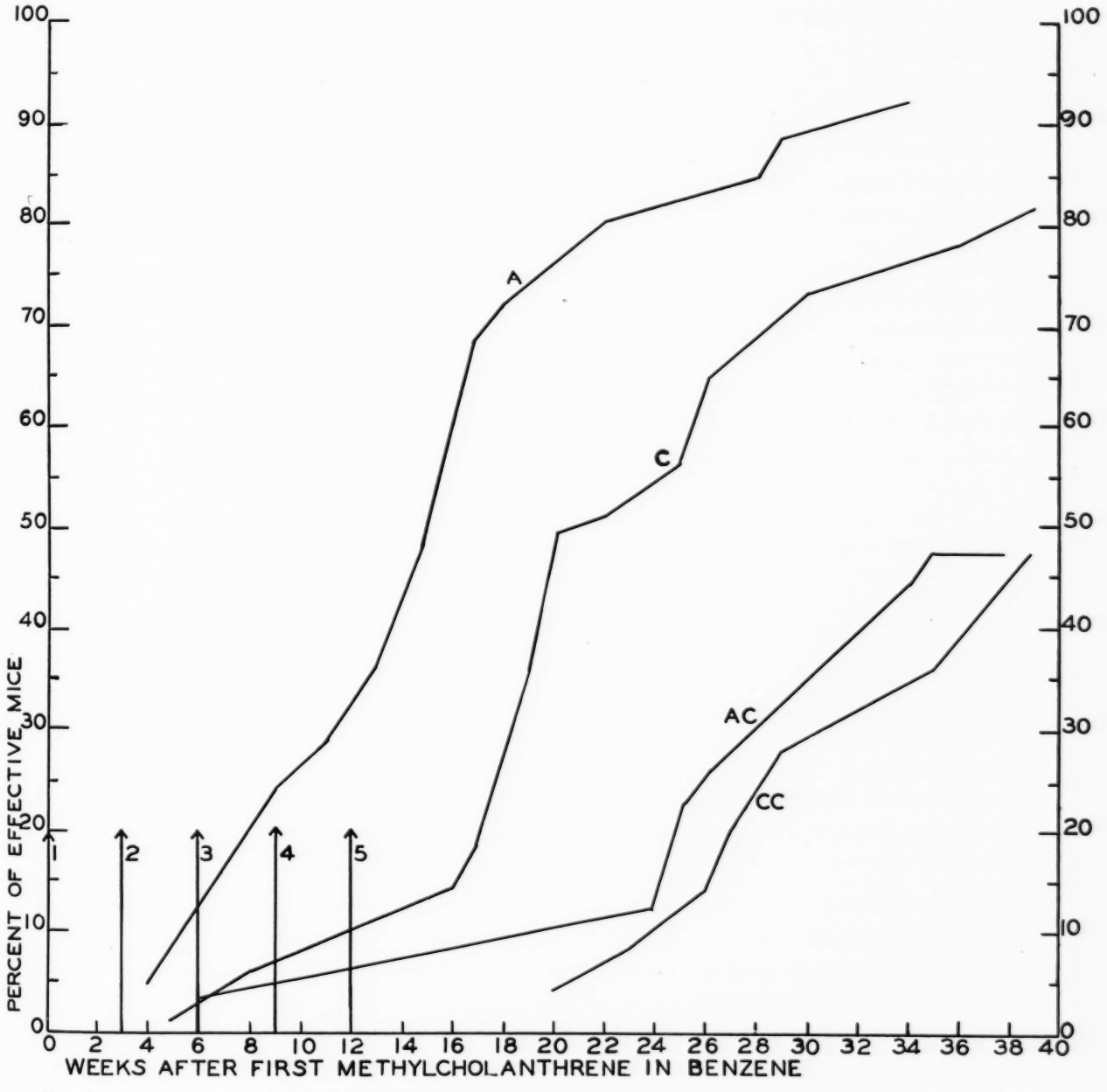


FIG. 1.—Comparison of carcinogenic response to 5 applications of 0.6 per cent methylcholanthrene in benzene by mice that had previously received 42 triweekly applications of 0.3 per cent methylcholanthrene in anhydrous lanolin (experiments A and C), and by previously untreated mice of the same age (control experiments AC and CC). Arrows indicate applications of carcinogen in benzene.

DISCUSSION

As a result of these experiments we may definitely rule out the possibility that the failure of a lanolin solution of methylcholanthrene to induce skin cancer is due to an increased resistance of the skin. The opposite is actually the case, as the accelerated carcinothrene itself. Some of the factors that may be involved will be discussed presently.

An additional finding of considerable interest is the fact that the lanolin solution of methylcholanthrene produces, without noticeably affecting the structure of the skin, a striking sensitization to subsequent car-

cinogenic response when the same carcinogen is applied in benzene solution.

This sensitization of the skin differs in one important point from other examples of sensitization recorded in the literature. By adding fresh liver to a basal ration Watson (12) obtained a greatly increased carcinogenic response in the skin of mice so far as the number of cancerous tumors was concerned, but the rate of development of malignancy was not increased. Bonser (2) inbred mice to produce a strain that also was more sensitive to carcinogens as measured by the number of cancerous skin tumors, but the skin cancers did not develop more rapidly in this strain. Estrinization of mice was found by Gilmour (6) to increase the carcinogenic response of the skin to benzpyrene so far as the number of carcinomas was concerned without, however, increasing the rate of their appearance. Paletta and Max confirmed these findings (7).

In our experiments there was not only a great increase in the number of carcinomas but also a pronounced acceleration of the carcinogenic response. In fact, the rate at which carcinomas appeared after the first application of the benzene solution of methylcholanthrene to the sensitized skins suggests that a considerable fraction of the tumors must have had their genesis as a direct and immediate response to that first exposure.

Returning now to the question why methylcholanthrene produces no carcinogenic effect when applied in lanolin solution, we find that possible explanations are still numerous, even though enhanced resistance of the skin has been eliminated.

First is the possibility that the carcinogen is inactivated as a result of a direct reaction with some constituent of anhydrous lanolin. On the basis of fluorescence spectrographic data this possibility appears unlikely. Characteristic methylcholanthrene spectra are still obtained from solutions of the carcinogen in lanolin that are now over 2 years old, even though this mixture was stored at room temperature and, for the many months during which experiments were in progress, the solution was heated on alternate days to 50° to 60° C. for an average period of an hour or so.

A second consideration may be that frequently advanced to explain the reduction of sarcogenic activity of injected carcinogens by lipids, that the inactivity of the lanolin solution results from the rapid absorption and excretion of the carcinogen in this readily absorbable solvent. Against this suggestion can be offered the evidence derived from fluorescence microscopic studies, that each of the 42 triweekly applications persists for at least 18 to 24 hours in the skin. An exposure of this magnitude should effect some morphological change if the methylcholanthrene is biologically active.

The fluorescence microscopic evidence cited above also rules out a third possibility, that the carcinogen, though not reactive with anhydrous lanolin *in vitro,* is rapidly and completely inactivated *in vivo* by a reaction with constituents of either the lanolin or of the tissue. The appearance in skin of the characteristic blue-violet fluorescence of methylcholanthrene for many hours after each exposure to lanolin solutions makes such an hypothesis untenable.

If we allow the facts to speak for themselves and dismiss preconceived ideas, a further possible explanation for the failure of a lanolin solution of methylcholanthrene to induce cancer is the assumption that methylcholanthrene is not, *per se,* carcinogenic. Such an assumption involves the separation of the actions usually ascribed to methylcholanthrene into two groups; those due to the unaltered hydrocarbon, and those due to some metabolic derivative. The experiments reported provide, for the first time, clear evidence that the different biological effects of a carcinogenic hydrocarbon may be separated. This suggests that the sensitizing action depends upon a chemical substance different from that or those producing the subsequent effects, such as epilation, destruction of sebaceous glands, epidermal hyperplasia, and finally, carcinogenesis itself.

Let us consider the last suggestion, that methylcholanthrene is not itself an active carcinogenic agent, in the light of these concepts. The role of unaltered methylcholanthrene would be to sensitize epidermal cells to the action of some of its metabolic derivatives, which, for brevity, we shall refer to as MCX. To MCX, which might actually represent a number of compounds, would be ascribed the initiation of all the subsequent activities that lead to cancer.

Several facts favor this explanation of the carcinogenic inactivity of lanolin solutions of methylcholanthrene. (a) Methylcholanthrene in lanolin does produce great sensitization of skin in the absence of other morphological effects. This occurs after the skin has been exposed to a great amount of unaltered carcinogen over a long period, since each of 42 applications persists for at least 18 to 24 hours. (b) Methylcholanthrene and the recorded constituents of anhydrous lanolin are, in general, relatively inert substances. In addition anhydrous lanolin contains powerful antioxidants, which would tend to prevent the oxidative breakdown of methylcholanthrene dissolved in it. (c) At no time did the skin, after having been painted with the methylcholanthrene-lanolin mixture, ever exhibit any fluorescence attributable to breakdown products of the carinogen. Conversely, such

fluorescences are regularly present in skin treated with the carcinogen in benzene (10).

In the preceding discussion an attempt has been made to analyze the remarkable lack of carcinogenic action of methylcholanthrene in anhydrous lanolin. The experiments have achieved their purpose in differentiating clearly between the skin and the carcinogen solution as the important factor in this phenomenon, but merely open up a larger vista of problems when it comes to the attempt to explain the specific nature of the state of methylcholanthrene. Some of the possibilities that may be involved have been suggested, and evidence that makes some of them less tenable than others has been presented. No final conclusions are permissible on the absolute basis of the available evidence. As a working hypothesis, however, the concept is advanced that unaltered methylcholanthrene is a sensitizing agent, itself noncarcinogenic, that prepares the skin for subsequent action by metabolic derivatives of the carcinogen, or by substances formed in the tissue as a result of exposure to these metabolic products. Attempts to test this hypothesis by animal experiments are now in progress. More direct evidence on the effect of metabolites of the carcinogenic hydrocarbons following the action of the unaltered carcinogen might be derived from a less complex situation in which a malignant change can be produced, such as has been so effectively used by Earle (5), but facilities are not available to us for this approach to the problem.

This concept of the mode of action of a carcinogenic hydrocarbon is not entirely new, nor does its sole support come from the evidence presented here. Earlier reports from this laboratory (3) have suggested that the direct effect of methylcholanthrene is a short toxic one, and that "subsequent epithelial proliferation can be accounted for adequately by assuming as a working hypothesis the formation in the skin of substances stimulating the cells to multiply over a prolonged period." These substances were designated as "proliferin." The results reported here support and expand such an hypothesis. It is now apparent that, at least under some conditions, methylcholanthrene has a sensitizing action and that its toxicity varies with the solvent. Possibly all the toxic effects are attributable to its decomposition products. Strength is given the major thesis, that methylcholanthrene is not, *per se,* responsible for inducing the specific cellular changes that lead, after a long interval, to the development of cancer.

For such a skin carcinogen as methylcholanthrene, and probably also for allied carcinogenic substances that are soluble in lipids and in lipid solvents, the manifestation of the carcinogenic effect, including the earliest changes initiating that effect, depends on the nature of the solvent. For convenience of description the group of solvents such as benzene, chloroform, and acetone, in which methylcholanthrene is carcinogenically active, will be described as forming "active" solutions; those in which methylcholanthrene is carcinogenically inactive will be referred to as making "inactive" solutions.

It appears, then, that a carcinogen in an inactive solution renders the skin more sensitive to the subsequent action of the same carcinogen when in an active solution. Forming one such inactive solution is sebum, a product of the skin that covers its outmost surface. The first effect of a carcinogen when applied in an active solution is to destroy the sebaceous glands. The glands remain intact when the carcinogen is applied in an inactive solution (8).

The existence of a chemical carcinogen for the skin in an inactive solution when dissolved in a secretion of the skin, and the sensitizing effect of this inactive solution, represent new factors in the etiology of skin cancer. They have some resemblance to allergic phenomena. Further investigations are necessary for the analysis of the significance of this phenomenon. But it may be pointed out that in the etiology of human skin cancer due to such agents as soot and tar, carcinogenic agents come into contact with the skin under conditions that do not necessarily involve removal of the sebum. These agents might, therefore, be dissolved in the sebum covering the surface of the skin and become inactive. But they might also sensitize the skin to the effects of a subsequent contact with a chemical carcinogen under conditions in which the protective action of the sebum is weakened or absent.

SUMMARY AND CONCLUSIONS

Methylcholanthrene, when dissolved in anhydrous lanolin, is rendered inactive in the sense that a prolonged administration of such a solution to mouse skin fails to induce those specific morphological and chemical changes that initiate the carcinogenic process, and regularly follow the application of methylcholanthrene dissolved in a lipid solvent such as benzene. A relatively simple chemical substance can, therefore, exist in a biologically active or inactive state according to the medium in which it is dissolved.

The inactive state is not due to lack of absorption of the methylcholanthrene by the skin. Nor can it be accounted for by postulating an increased resistance of the skin to the action of the carcinogen, for treatment with "inactive" methylcholanthrene greatly increases the sensitivity of the skin to a subsequent application of the carcinogen in the "active" state; *i.e.,* when dissolved in benzene. This increased sensitivity manifests itself in a great increase in the incidence of

skin cancer, and a great shortening of its induction period.

The significance of these findings in relation to a concept of a mode of carcinogenic action of methylcholanthrene is discussed.

REFERENCES

1. Berenblum, I. The Carcinogenic Action of Croton Resin. Cancer Research, **1**:44-48. 1941.
2. Bonser, G. M. The Hereditary Factor in Induced Skin Tumours in Mice. Establishment of a Strain Especially Sensitive to Carcinogenic Agents Applied to the Skin. J. Path. & Bact., **46**:581-602. 1938.
3. Cramer, W., and Stowell, R. E. Carcinogenesis in the Mouse's Skin by the Infrequent Application at Long Intervals of Methylcholanthrene. Cancer Research, **1**: 849-852. 1941.
4. Cramer, W., and Stowell, R. E. On the Quantitative Evaluation of Experimental Skin Carcinogenesis by Methylcholanthrene. The Factors of Dosage, Time, Spacing of Applications, and the Multiplicity of the Carcinogenic Response. Cancer Research, **3**:668-681. 1943.
5. Earle, W. R. Production of Malignancy in Vitro. IV. The Mouse Fibroblast Cultures and Changes Seen in the Living Cells. J. Nat. Cancer Inst., **4**:165-212. 1943.
6. Gilmour, M. D. An Investigation into the Influence of Oestrone on the Growth and on the Genesis of Malignant Cells. J. Path. & Bact., **45**:179-188. 1937.
7. Paletta, F. X., and Max, P. F. Influence of Estradiol Benzoate on Epidermal Methylcholanthrene Carcinogenesis. J. Nat. Cancer Inst., **2**:577-581. 1942.
8. Simpson, W. L., and Cramer, W. Sebaceous Glands and Experimental Skin Carcinogenesis in Mice. Cancer Research, **3**:515-518. 1943.
9. Simpson, W. L., Carruthers, C., and Cramer, W. Loss of Carcinogenic Activity when Methylcholanthrene is Dissolved in Anhydrous Lanolin. Cancer Research, **5**: 1-4. 1945.
10. Simpson, W. L., and Cramer, W. Fluorescence Studies of Carcinogens in Skin. II. Fluorescence Studies of Mouse Skin after Repeated Applications of 20-Methylcholanthrene. In preparation.
11. Twort, C. C., and Twort, J. M. Studien über Krebsentstehung. Krebsbildungsfähigkeit. Ztschr. f. Krebsforsch., **32**:491-519. 1930.
12. Watson, A. F. Tar Cancer in Mice Maintained on Diets Supplemented with Fresh Liver. Am. J. Cancer, **19**: 389-395. 1933.

Carcinogenesis Induced by Methylcholanthrene in Pigeons, Guinea Fowls, and Ducks*

F. Duran-Reynals, M.D., E. W. Shrigley, Ph.D., M.D.,** and E. de Hostos, M.D.***

(From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication July 22, 1944)

While the induction of malignant tumors in chickens by chemical carcinogens has been achieved by several workers, carcinogenesis in other species of birds has not been reported. The present article describes several sarcomas, some of them serially transplantable, induced in pigeons, guinea fowls, and in 1 duck by injections of methylcholanthrene.

Table I: Carcinogenesis in Pigeons by Methylcholanthrene

Exper. No.	Pigeon No.	Total carcinogen injected	Spacing of injections	Effect	Tumor first noticed, mos.	Bird killed, mos.	Metastases	Transplantability and number pigeons injected
I	1	12 mgm. in 0.4 cc. benzol	Single injection	Tumor 4.0 × 2.3 cm.	3 to 4	4½	Bones and marrow of lower extremities	Negative. 3
	2	"	"	" microscopic		4½	Absent	Not attempted
	3	"	"	" 3.7 × 2.5 cm.	3 " 4	4½	"	Negative. 2
	4	"	"	" 5.5 × 4.0 "	2 " 3	4½	Liver	" 3
	5	"	"	" 2.2 × 2.0 "	2 " 3	5	"	Positive, 1 of 3
	6	"	"	" 4.0 × 3.0 "	2 " 3	3½	" bone marrow, lower extremities	" 1 of 3
	7, 8, 9	"	"	" none		4½		
II	10	15 mgm. in 1.5 cc. benzol	First injection 5 mgm.; second, 10 mgm. 4 mos. later	" 3.0 × 2.5 "	6 " 7	8	Liver	Negative. 4
	11	"	"	" 4.0 × 3.0 "	6 " 7	8	Absent	Positive, 1 of 5
	12	"	"	" 1.0 × 1.0 "	7 " 8	10½	"	Negative. 2
	13, 14, 15, 16	"	"	" none				
III	17	10 mgm. in 1 cc. benzol	20 injections of 0.05 cc. in 24 days	" 2.0 × 2.0 "	4 " 5	9	"	" 3
	18	"	"	" 2.5 × 1.5 "	4 " 5	6½	Liver	" 3
IV	19	20 mgm. in 2 cc. benzol	20 injections of 0.1 cc. in 24 days	" probably, later regressed				
	20	"	"	Tumor probably later regressed				
	21			Tumor, none				

Experiments on Pigeons

The study started about 4 years ago when pigeons were injected in the breast with 4 mgm. each of methylcholanthrene dissolved in lard. No gross tumors developed in the next few months, but when the birds were killed histological examination showed in several of them the presence of nests of typical sarcoma cells interspersed in the very conspicuous fibrous reaction that had been elicited by the carcinogen.

In view of the superiority of benzol solutions of the carcinogen as compared with lard solutions for inducing local malignancy in fowls (3), the study was repeated by injecting 1 per cent and 3 per cent solutions of methylcholanthrene in benzol into the pectoral muscles of pigeons of from 3 to 6 months of age. Four experiments were carried out on 35 pigeons from the usual strains found in poultry markets. Before and after the injection of the chemical the birds were kept together in a large cage, but in the same room where other animals, among them chickens, guinea fowls, and ducks bearing infectious tumors, were kept. The results and details of the experiments are given in Table I.

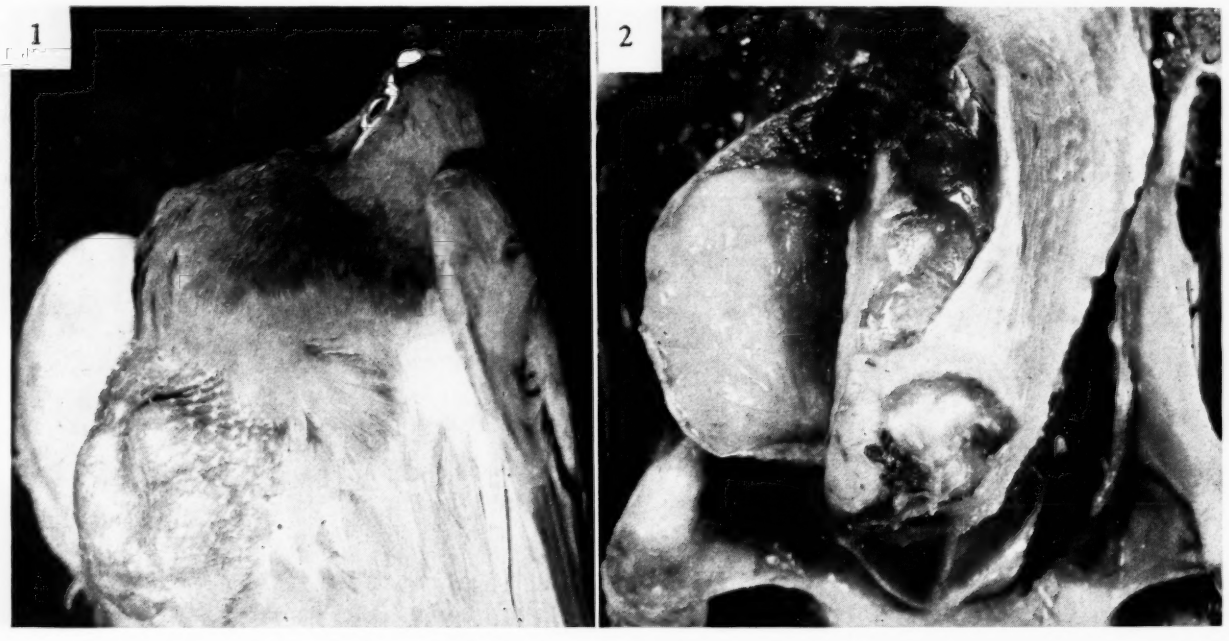
It is seen in the table that 10 progressively growing tumors developed in the 21 pigeons injected (47 per

* This investigation was aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research and The International Cancer Research Foundation.

** Fellow of The International Cancer Research Foundation.

*** A part of this study was used by Dr. de Hostos as material for his thesis in partial fulfillment of the requirements for the degree of Doctor of Medicine, Yale University School of Medicine, 1942.

cent), while a microscopic tumor was found in another bird. In the last 3 experiments 14 more pigeons were injected with from 12 to 20 mgm. of methylcholanthrene, but all died 2 to 4 months after the first injection of the carcinogen, either of intercurrent diseases or of acute benzol poisoning. Since by that time none of the other pigeons in the same experiments had developed tumors they have not been included in the calculations. The table shows, also, that the highest incidence and earliest development of tumors were obtained in Experiment 1, where the carcinogen, dissolved in a small amount of solvent, was given in 1 injection.



FIGS. 1 and 2.—Sarcoma induced in pigeons by 12 mgm. of methylcholanthrene, 5 months after injection of the carcinogen. This growth was the original of tumor 1, kept for 6 passages through pigeons.

The tumors originating at the site of injection varied in size, as shown in the table, and ranged in consistency from pronounced hardness to extreme softness, sometimes accompanied by viscidity. They were well demarcated from the surrounding muscle and only occasionally did they invade the skin and become ulcerated (Figs. 1 and 2). A variable amount of central necrosis was not infrequent.

In 2 pigeons of Experiment 4 soft masses were felt from 4 to 5 months after injection, which grew in the few months following until they attained a size of 4×1.5 cm. Later they disappeared, but as no biopsies had been made the neoplastic nature of these growths, although probable, was not demonstrated.

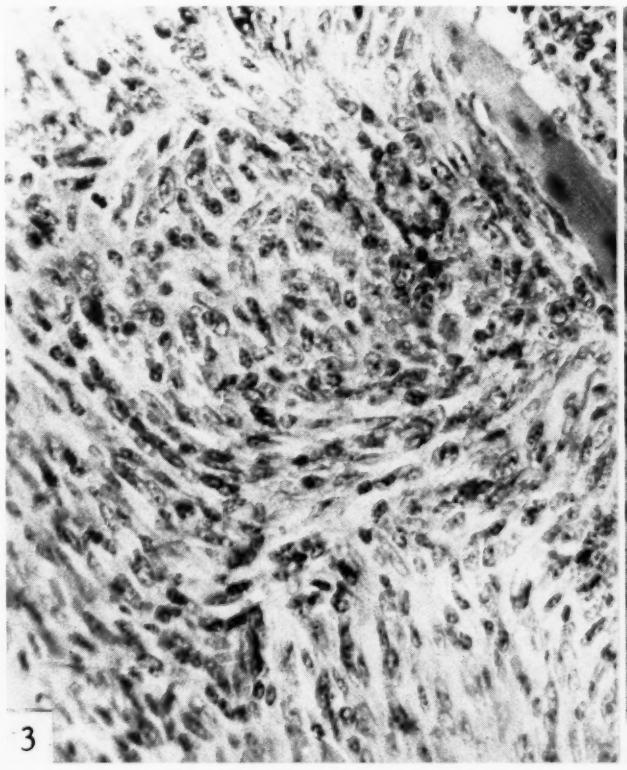
All the tumors examined microscopically were sarcomas (Figs. 3 and 4). The hard, compact growths were made up of packed, attenuated fibroblasts, often with numerous mitoses, whereas the soft growths consisted of round, often distorted cells, loosely arranged and showing few mitoses. Thus the latter growths came to resemble transplantable chicken tumors of the Rous sarcoma type. Collagen was scant. Collections of lymphoid cells were sometimes observed around and in the tumor tissue.

As is shown in the table, metastases were observed in about half of the birds showing tumors, and their tendency to develop in the long bones, as observed in 2 cases, seems to be the only characteristic of interest (Fig. 5). In general the histological traits of the main tumor were reproduced in its metastases. In the liver, perivascular clusters of lymphoid and myeloid cells were sometimes observed, but no other systemic changes were noticed.

The pigeons that died before attaining the age for tumor development showed at the injected site muscle necrosis accompanied by considerable fibrosis, which in some areas gave the impression of malignancy. Nodules of lymphoid cells were again observed in some of the lesions.

Transplantation of the gross tumors was attempted in all cases by injecting into the breast muscle, and occasionally into the peritoneum, of pigeons 2 cc. of a suspension of 1 gm. of tumor in 5 cc. of physiological saline solution. As indicated in the table, growths were obtained from 3 of the tumors.

The tumor from pigeon No. 11 apparently grew in 2 of 5 pigeons grafted, but the growths regressed



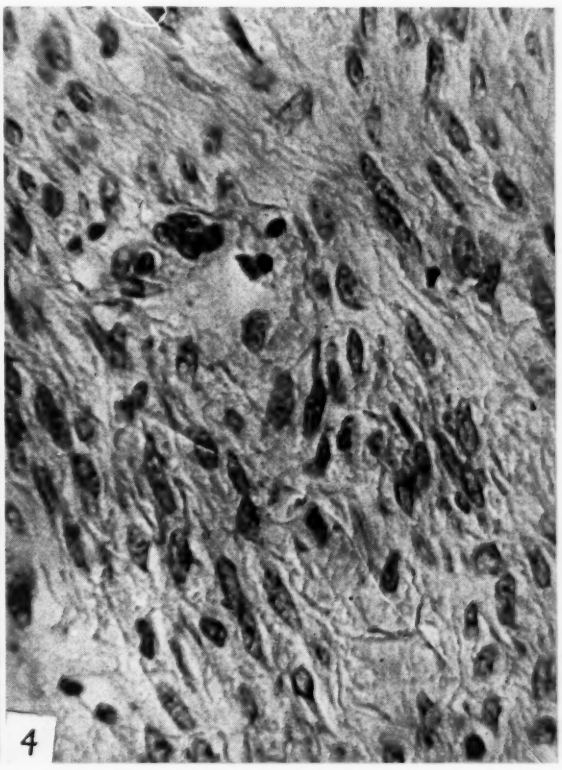


FIG. 3.—Primary tumor in pigeon No. 11 killed 8 months after the injection of 15 mgm. of methylcholanthrene. Mag. × 300.
FIG. 4.—Primary tumor induced in pigeon No. 5 by 12 mgm. of methylcholanthrene, 5 months after injection of the carcinogen. Mag. × 600.

FIG. 5.—Metastatic periosteal tumor in pigeon No. 1, killed 4½ months after the injection of 12 mgm. of methylcholanthrene. Mag. × 80.

later. The tumor of pigeon No. 5 (pigeon tumor 1) was transplanted through 6 successive generations of pigeons, and tumors developed in 12 of the 28 birds injected. The strain was lost when a seventh passage was attempted. The speed of growth of the transplanted tumors was variable. Some attained a size of 4 × 2 cm. in less than 2 months, whereas others were far smaller after 150 days. Six of the pigeons died of their tumors in from 50 to 150 days after inoculation, and liver metastases were found in 3 of them at the second passage, the passage in which the tumor grew best (Fig. 6). The other 5 pigeons, killed to provide transplantation material, showed no metastases. The growths were soft, not viscid, and generally contained little necrotic tissue.

The tumor of pigeon No. 6 (pigeon tumor 2) has been passed through 9 generations of pigeons and is still being transplanted. It grew in 40 of 62 pigeons injected, often attaining a large size, but as it had a definite tendency to become necrotic the amount of living tissue found was often negligible. The original tumor was sterile, soft, and not viscid, but in the course of transplantation the tumor became infected and extremely viscid. Sterile tumors were secured later by the injection of very dilute tumor-cell suspensions, and these growths, although soft, were free of necrosis and showed very little or no viscidity. The

injected pigeons died between 1 and 2 months after inoculation. Metastases were never observed (Fig. 7).

In general the transplanted tumors from both strains, 1 and 2, rather faithfully reproduced the features of the original neoplasms, which were hard and compact (Figs. 8 and 10). Occasionally, though, the transplants were of loose texture (Fig. 9).

Transmission by Berkefeld N filtrates was attempted with extracts of both these growths at different passages, 5 adult pigeons being injected with tumor 1, and 21 with tumor 2. The birds were inoculated in both vein and muscle, sometimes with as much as 10 cc. of filtrate diluted 1:20. The results have been negative.

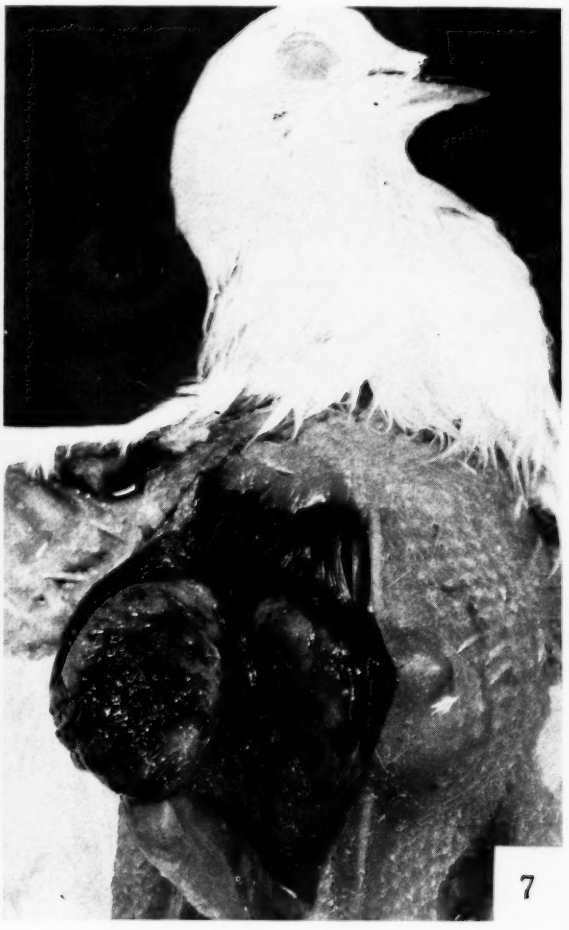


FIG. 6.—Primary tumors and liver metastases in a pigeon of the second passage of tumor 1.
FIG. 7.—Pigeon tumor 2, eighth passage.

EXPERIMENTS ON GUINEA FOWLS

Twelve guinea fowls ranging in age from 6 to 12 months were injected intramuscularly in the breast with either 15 or 20 mgm. of methylcholanthrene dissolved in benzol, as indicated in Table II. The carcinogen was administered in 2 doses with an interval of 40 days, the first injection consisting of either 5 or 10 mgm. and the second of 10 mgm. The birds were housed under the conditions described for the pigeons.

Tumors developed in 8 of the 12 guinea fowls (66 per cent) at the site of injection. They were all firm except where the growth perforated the skin, a rather frequent event (Figs. 11, 12, 13). They were all spindle cell sarcomas, compact, very collagenous, and consisting of attenuated fibroblasts showing either few or a fair number of mitoses (Fig. 14). One of them, however, that of guinea fowl No. 10, was composed of large, distorted cells, loosely arranged, and had abundant intercellular material, but little collagen. Metastases, always in the liver, were observed in 3 cases (Fig. 15).

Transplantation by means of cell suspensions was attempted in 6 instances, positive results being obtained in 1 case, tumor 12, which was successfully passed through 2 generations of guinea fowls. The tumor grew in 1 of the 2 guinea fowls of the third

passage, but regressed later. The transplanted tumors sometimes attained a size of 3×2 cm. in 15 days, and of 15×9 cm. in 3 months, but produced no metastases. Histologically they looked like the original tumor. The scarcity of birds prevented more extensive transplantation tests and transmission by filtrates could not be attempted for the same reason.

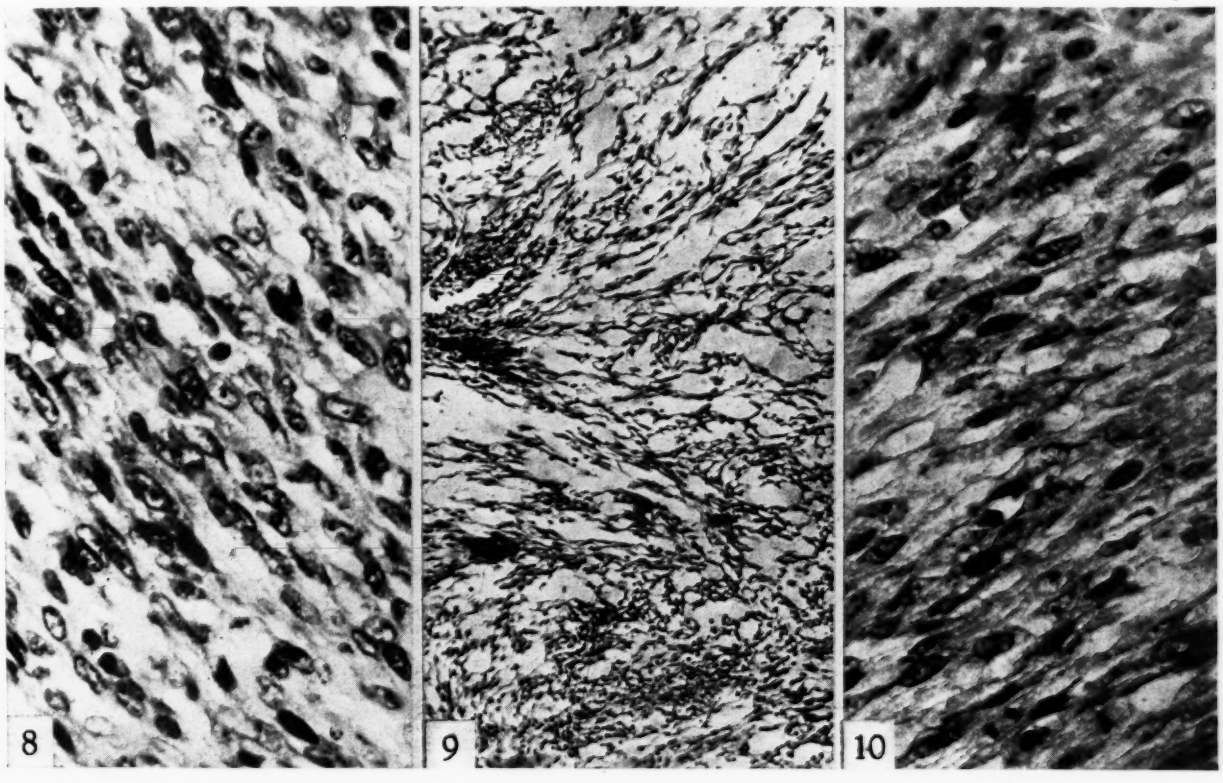


FIG. 8.—Sarcoma induced in pigeon No. 11 by 15 mgm. of methylcholanthrene, 8 months after injection of the carcinogen. This growth was the original of tumor 2. Mag. $\times$ 725.

FIG. 9.—Pigeon tumor 2, ninth passage. Note loose texture. Mag. $\times$ 120.

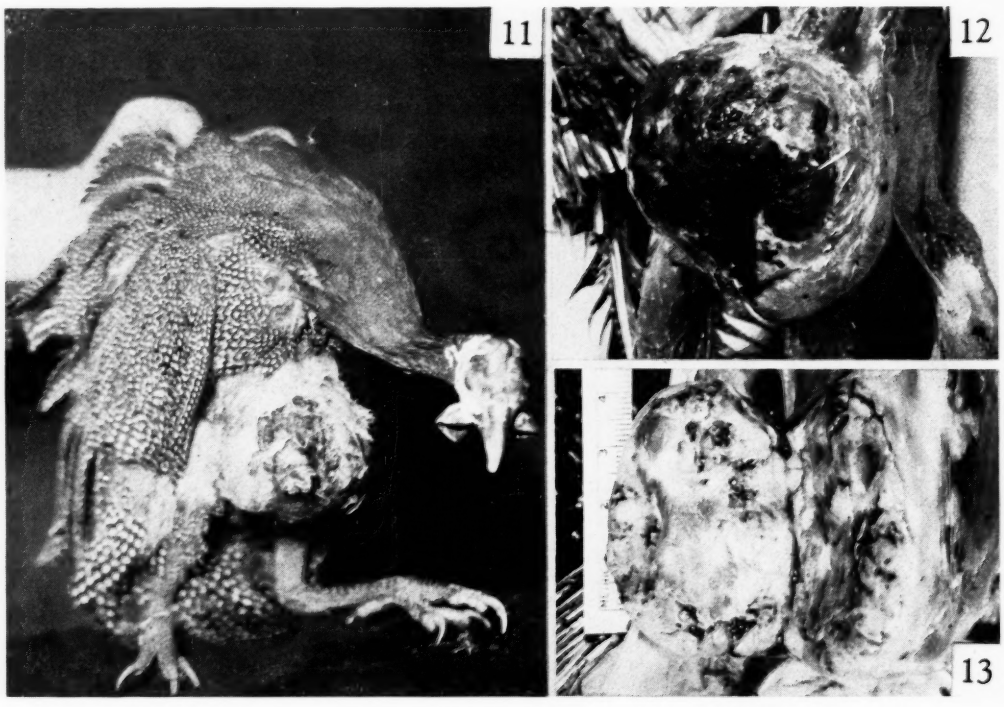
FIG. 10.—Pigeon tumor 2, seventh passage. Note compact texture as in the original tumor. Mag. $\times$ 600.

TABLE II: CARCINOGENESIS IN GUINEA FOWLS BY METHYLCHOLANTHRENE

Guinea fowl No.	Total carcinogen injected	Effect	Tumor first noticed, mos.	Bird killed or died, mos.	Metastases	Transplantability and number guinea fowls injected
1	15 mgm. in 1.5 cc. benzol	Microscopic tumor		9		
2	"	Tumor 4.0 × 3.0 cm.	4 to 5		Absent	Negative. 2
3	"	" none		9		
4	"	Probable tumor that later regressed		9		
5	"	Tumor, none	7 " 8	7 (died)		
6	20 mgm. in 2 cc. benzol	" "		10 "		
7	"	" 8.0 × 7.0 cm.	4 " 5	10 "	Liver	" 3
8	"	" 10 × 6 "	3 " 4	10 "	"	" 2
9	"	" 5.0 × 3.8 "	6 " 7	7	Absent	" 2
10	"	" 5.0 × 3.0 "	6 " 7	7	"	" 2
		" 3.7 × 2.5 "				
11	"	" 8.0 × 7.0 "	6 " 7	12	Liver	Not attempted
12	"	" 5.0 × 4.5 "	2 " 3	4	Absent	Positive in all 5

EXPERIMENTS ON DUCKS

In the first series of experiments 45 Pekin ducks ranging in age from 1 day to 8 months were injected in the breast muscles with 8 mgm. of methylcholanthrene dissolved in lard for the birds 1 to 16 days old, and with 24 mgm. for the older birds. Many of these ducks were also injected intravenously with the virus of the Rous sarcoma, in order to study the joint effects of the virus and methylcholanthrene in birds more than 1 day old. It is known that only ducks of 1 day or less are susceptible to the Rous virus (1). The virus, either in filtrate or cell suspensions, was injected at the same time or at different periods before or after the injection of carcinogen, and the birds



FIGS. 11, 12, and 13.—Tumors induced in guinea fowls Nos. 11, 9, and 2 by 15 or 20 mgm. of methylcholanthrene, 12, 7, and 5 months respectively after injection of the carcinogen.

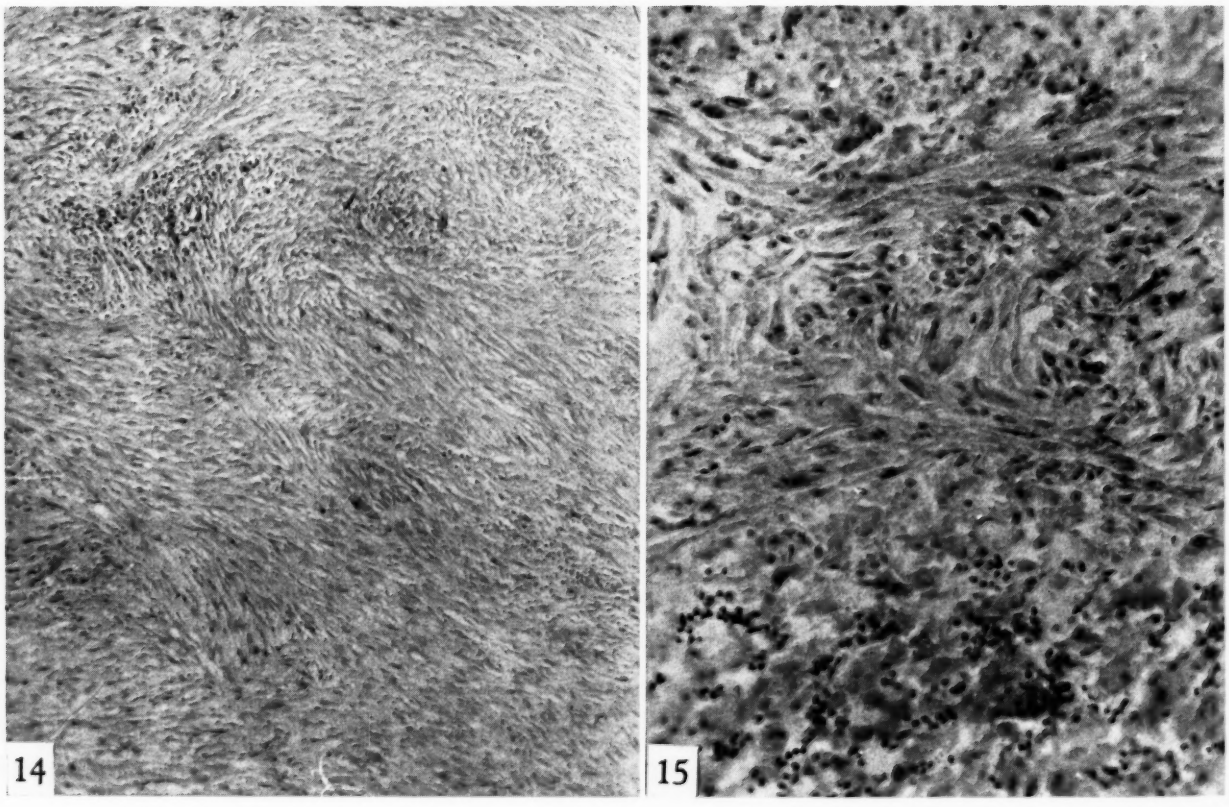


FIG. 14.—Sarcoma induced in guinea fowl No. 2. Mag. × 120.
FIG. 15.—Liver metastases in guinea fowl No. 8. Mag. × 335.

were kept for from 7 to 10 months after the methylcholanthrene was injected. In no case did tumors develop, and the inoculation of newborn ducks with suspicious lesions found at autopsy always gave negative results.

In a second experiment 7 Pekin ducks 7 days old were injected in the breast muscles with 10 mgm. of methylcholanthrene dissolved in 1 cc. of benzol, and this procedure was repeated until a total of 40 mgm. had been introduced over a period of 6 months. The birds were observed for 12 months after the first injection. In 1 of them an induration 6×4 cm. was found at the injection site 9 months after the first injection, but biopsy showed only necrotic matter, which was removed. A month later the region became indurated again, and when the bird was killed after another 2 months a tumor 2.7×1.5 cm., firm and free of necrosis, was found. Microscopic examination showed the growth to be a fibrosarcoma. The skin over a large area around the tumor appeared thickened and edematous, indicating an infection of long standing, and cultures from both the tumor and the surrounding tissues yielded a variety of bacterial colonies. Cell suspensions of the tumor were injected into seven 2 day old ducklings, but no growth was obtained and the birds were killed 4 months later.

Experiments with Turkeys

Five turkeys, 2 weeks old, were injected with 2 mgm. of methylcholanthrene in 0.2 cc. of benzol in the muscles of the breast. Six months later the birds were given 10 mgm. of the carcinogen in 1 cc. of benzol, and after another 2 months 10 mgm. more were similarly injected.

Two other turkeys were injected in the same way with 30 mgm. of the chemical, spaced in 3 injections of 10 mgm. each over a period of 8 months. The birds died or were killed from 6 to 10 months after the first injection, but in no case had tumors or other lesions developed except an occasional fibrous and lymphoid reaction at the injection site.

Three other turkeys injected with 2 mgm. and 1 with 10 mgm. of methylcholanthrene died in from 3 to 4 months without lesions.

Comment and Summary

It appears from the experiments that pigeons, guinea fowls, and possibly ducks may be added to the list of animals responding to the injection of methylcholanthrene with malignant tumors. The incidence in 21 pigeons was 47 per cent and in 12 guinea fowls 66 per cent, whereas only one tumor developed in 52 ducks and none in 10 turkeys injected.

The results show that there is no species relationship between the susceptibility of birds to the virus of the Rous sarcoma (1, 2) and to methylcholanthrene.

Transplantation of 2 pigeon and 1 guinea fowl tumor through from 2 to 9 generations of birds of their respective species was accomplished, but attempts to transmit the pigeon tumors by means of Berkefeld N filtrates ended in failure. Filtration of the guinea fowl tumor was not done.

All the tumors induced were ordinary spindle cell sarcomas that frequently gave rise to metastases in the liver and, in the case of 2 pigeon tumors, in the bones of the lower extremities.

Benzol solutions of methylcholanthrene proved to be far more effective than lard solutions.

References

1. Duran-Reynals, F. The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. Cancer Research **3**:569-577. 1943.
2. Duran-Reynals, F. The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses. Cancer Research, **2**:343-369. 1942.
3. Murphy, J. B., and Sturm, E. Further Investigation of Induced Tumors in Fowls. Cancer Research, **1**:477-483. 1941.

Biocatalysts in Cancer Tissue

V. Adenosinetriphosphatase*

Van R. Potter, Ph.D., and Gertrude J. Liebl, B.S.

(From the McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison 6, Wisconsin)

(Received for publication August 14, 1944)

In previous publications a theory of cancer has been presented (5, 6) in which its uncontrolled growth was explained on the basis of a deficiency in respiratory enzymes, which esterify inorganic phosphate, as compared with the energy-depleting enzymes, such as those that hydrolyze phosphate esters. It was proposed that an imbalance between the respective enzymes produces an increase in the concentration of inorganic phosphate, adenosine, and other essential building blocks needed for the growth process. This theory would account for the high glycolysis of tumor tissue on the same basis.

Assays for specific components of the respiratory enzyme systems have revealed that, in comparison with specialized adult tissues, neoplasms are deficient in cytochrome *c* (1), succinic dehydrogenase (8), and cytochrome oxidase (8). This observation also applied when hepatomas were compared with normal liver.

With the development of a method for the assay of adenosinetriphosphatase in small tissue samples (2), it became possible to determine quantitatively the concentration of this enzyme in tumors. The results show that, in contrast to the respiratory enzymes studied, ATP-ase is present in neoplasms in amounts comparable to those present in specialized adult rat tissues. The method was used exactly as described (2), and applied to a series of tumors as in the case of previous studies in this series (1, 8).

TABLE I: ATP-ASE CONTENT OF EXPERIMENTAL TUMORS COMPARED WITH NORMAL RAT TISSUES (2)

Results expressed in ATP-ase units: Micrograms of inorganic P split off per mgm. of fresh tissue in 15 minutes

	Tumor tissues					Normal tissues with calcium	
		No calcium, average	With calcium				
Type of tumor	No. of samples		Max.	Min.	Aver.	Tissue	Average
Jensen rat sarcoma	9	2.0	29.0	14.3	20.2	Heart muscle	27.3
U. V. tumor, mouse (transplants)	9	3.5	27.2	11.3	19.3	Skeletal muscle	23.3
						Lung	21.8
						Kidney	20.3
Spontaneous mammary tumors	3	2.9	15.2	11.8	13.3	Submaxillary gland	16.4
Dietary liver tumors (*p*-dimethyl-aminoazobenzene)	10	1.9	15.8	8.5	11.8	Spleen	13.0
						Liver	12.9
						Pancreas	11.5
Walker rat carcinosarcoma 256	5	1.6	19.7	6.9	11.5	Smooth muscle	8.2
						Brain	7.0
Flexner-Jobling rat carcinoma	8	1.3	11.1	4.0	8.0		
Yale mouse No. 1	12	1.6	10.6	2.6	5.6		

RESULTS

The results of the analyses of 56 samples of tumor tissues, representing 7 different types of experimental neoplasms, are shown in Table I, in which the tumors are listed in the order of their ATP-ase activity. Values for normal rat tissues are listed in the same manner in the table. The maximum value for normal tissues is 27.3 for heart, and the minimum for the tissues observed was 7.0. In the case of the neoplastic tissues the maximum was 20.2, and the lowest value was 5.6. In the only instances where comparison is possible on the basis of homology, the liver tumors contained an average of 11.8, in comparison with a normal liver value of 12.9. This difference is not considered significant. Thus the neoplasms contained, in general, an amount of ATP-ase not significantly different from adult specialized tis-

* This work was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

sues. This seems to be particularly significant in view of the observation (7) that in the case of brain and liver, at least, the tissues of the newborn rat contained only one-third to one-sixth as much ATP-ase as the adult tissues. In these tissues the succinoxidase components were also low, in comparison with the adult tissues. Thus the tumor tissues appear to resemble the adult tissues with respect to ATP-ase, and to resemble less differentiated tissue with respect to the succinoxidase components.

The data show that the ATP-ase in tumor tissues is activated by calcium ions, and is in this respect comparable to the ATP-ase of normal tissues (2).

DISCUSSION

The measurements presented in this report record the *potential* ATP-ase activity of neoplastic tissues as compared with normal ones. They do not contribute to our knowledge of the *effective* concentration of ATP-ase in these tissues. Nevertheless they are valuable, since they prove that tumors are not simply a tissue type in which all the energy-mobilizing enzymes are low, for it is shown that the tumor tissues have an ATP-ase content of about the same magnitude as the specialized adult tissues, in contrast with the results for the respiratory enzymes previously studied (1, 8).

The problem of measuring the *effective* concentration of the individual enzymes in neoplastic and normal tissues must remain unsolved for the present. However, the resultant of the effective concentrations of all the energy-mobilizing enzymes can be appraised by determining the concentration of certain strategic metabolites in tissues that have been frozen *in situ* and analyzed by the technics of LePage and Umbreit (3). Studies in which normal tissues have been analyzed by these technics have shown (4) that in normal tissues the splitting of the ATP is greatly accelerated following stimulation of the respective tissues by extrinsic factors, which are apparently nerve impulses. From this it may be inferred that the effective concentration of ATP-ase, that is, ATP-ase activity, is low in normal tissues at rest, and that in these tissues the substrate (ATP) concentration does not determine the ATP-ase activity, since the ATP content is maximal in resting tissues. On the other hand, it is generally assumed that tumors are unresponsive to the extrinsic factors that control activity in normal tissues; if this is true, one might expect the activity of ATP-ase to be regulated in neoplasms by the concentration of ATP to a greater extent than is true for normal tissue. Thus the *effective* concentration of ATP-ase can be greatly altered, depending on the prevailing concentration of its substrate, ATP, among other things, and its activating co-factor, the calcium ion. The potential activity is measured with these factors at the optimum concentration.

The exact use to which the energy of ATP is put when ATP-ase acts in tumor tissue is still unknown, just as it is in all other tissues except muscle. It is particularly interesting to find that the ATP-ase of neoplastic tissue is activated by calcium ions, just as in the case of normal tissues (2). The fact that certain tumors contain less calcium than normal tissues (10) is probably related to ATP-ase activity, but it does not follow that ATP-ase activity will be less because the calcium content is lower. Indeed, the opposite might be inferred, on the basis of the fact that a lowering of the concentration of serum calcium increases nerve irritability (9). An appraisal of the state of the calcium ions in tumor tissue is needed.

It is apparent that the complete description of the difference between normal tissues and tumors will require considerable time. However, the broad outlines of this difference are already apparent, and the results to date indicate that discernible differences do exist. Even if one assumed that the activity coefficients of ATP-ase, succinic, dehydrogenase, and cytochrome oxidase were the same in normal and in tumor tissue, a shift to the right in the balance between ATP and its breakdown products would be suggested for neoplastic tissue. The possibility of a greater activity coefficient for ATP-ase in tumors than in normal tissue has been discussed above, and would increase the difference between neoplasms and adult, specialized, normal tissue. The balance in the case of growing normal tissue would not necessarily be different from tumor tissue except in its ability to develop an ATP-balance that characterizes adult tissue (5-7).

SUMMARY

1. Seven types of experimental tumors were assayed for their ATP-ase content. They were found to contain about the same amount as adult specialized tissues, in contrast to the findings for various respiratory enzymes.

2. It is pointed out that the assay measures potential ATP-ase rather than the *effective* ATP-ase concentration of the tissues. The factors that determine the effective ATP-ase concentration in normal and tumor tissue are discussed, and it is suggested that the activity coefficient in neoplasms is likely to be at least as great as in normal tissue.

3. The data are discussed in relation to the theory of cancer that relates growth to the balance between ATP and its breakdown products.

REFERENCES

1. DuBois, K. P., and Potter, V. R. Biocatalysts in Cancer Tissue. I. Cytochrome *c*. Cancer Research, **2**:290-293. 1942.
2. DuBois, K. P., and Potter, V. R. The Assay of Animal Tissues for Respiratory Enzymes. III. Adenosinetriphosphatase. J. Biol. Chem., **150**:185-195. 1943.
3. LePage, G. A., and Umbreit, W. W. In Umbreit, W. W. Manometric Methods and Related Techniques for the Study of Tissue Metabolism. Minneapolis: 1944. In press.
4. LePage, G. A. In press.
5. Potter, V. R. Biocatalysts in Cancer Tissue. IV. An Enzyme-Virus Theory Regarding Carcinogenesis. Cancer Research, **3**:358-361. 1943.
6. Potter, V. R. Biological Energy Transformations and The Cancer Problem. In Nord, F. F., and Werkman, C. H. Advances in Enzymology, Vol. IV, New York: Interscience Publishers, 1944, pp. 201-256.
7. Potter, V. R., Schneider, W. C., and Liebl, G. J. Enzyme Changes During Growth and Differentiation in the Tissues of the Newborn Rat. Cancer Research, **5**: 21-24. 1945.
8. Schneider, W. C., and Potter, V. R. Biocatalysts in Cancer Tissue. III. Succinic Dehydrogenase and Cytochrome Oxidase. Cancer Research, **3**:353-357. 1943.
9. Solandt, D. Y. Measurement of "Accommodation" in Nerve. Proc. Roy. Soc., London, s. B, **119**:355-397. 1936.
10. Suntzeff, V., and Carruthers, C. The Effect of Methylcholanthrene upon Epidermal Sodium and Calcium. Cancer Research, **3**:431-433. 1943.

Enzyme Changes During Growth and Differentiation in the Tissues of the Newborn Rat*†

Van R. Potter, Ph.D., W. C. Schneider, B. S., and Gertrude J. Liebl, B.S.

(From the McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison 6, Wisconsin)

(Received for publication August 14, 1944)

A theory of cancer has been presented (10, 11) in which it was suggested that growth may be correlated with a lowering in the effective concentration of a hypothetical enzyme X, which was suggested to be a respiratory enzyme or a complex of respiratory enzymes (11). According to this theory, growth in specialized tissues is prevented by an intrinsic scarcity of factors essential to it. Available evidence as to the identity of these essential growth factors emphasizes the breakdown products of the reservoirs of phosphate-bond energy, and it was pointed out that the concentration of these products is the resultant of the balance between the energy-requiring processes of function and maintenance versus the activity of the energy-yielding processes of glycolysis and respiration (11).

A corollary of the theory is that tumor tissue and growing normal tissue should contain less of the respiratory enzymes than specialized adult tissues, and there is no reason, *a priori,* why tumor tissue should contain any less of these enzymes than growing undifferentiated tissue. However, growing tissue with concomitant specialized function might have a higher concentration of the energy-providing respiratory enzymes than tumor tissue, if at the same time the concentration of the energy-depleting functional enzymes were increased as well. The problem of identifying and testing for an enzyme of the latter class is a difficult one, but recent work has shown that the ATP-ase of muscle is one of the best representatives of this type of enzyme available. Although the function of ATP-ase in tissues other than muscle remains obscure, it is present in high concentrations in adult specialized tissues; and all the tissues examined contained ATP-ase, which was activated by calcium ions just as is the case in muscle (4).

In order to test the hypothesis of growth control it is necessary to have growing tissue as well as enzyme methods that can be applied to small samples of tissue. The tissues of the newborn rat seemed to offer possibilities as a source of growing tissue. Himwich and his co-workers (5, 6), as well as numerous others, have shown that the newborn rat is highly resistant to anoxia in comparison with the adult, and that in the course of 10 to 20 days it loses this resistance and becomes as susceptible as the adult. Resistance to anoxia was inversely correlated with the oxygen uptake rate of samples of cerebral tissue (5), and was suggested to be due to low functional activity. The newborn rat brain has been stated to be physiologically as young as that of the pig embryo between the 50 mm. and 100 mm. stage. Koch suggested that the newborn rat's brain is "as young nervous material as can conveniently be analyzed at present; and it forms, therefore, a convenient starting point for the study of the chemical differentiation of the central nervous system during growth" (7). The resistance of the newborn rat to anoxia suggests a relative independence from aerobic processes in comparison with adult specialized tissue. In this respect it is similar not only to embryonic tissue (9) but to cancer tissue as well (8). The fact that the newborn rat brain is embryonic in type, and that the conversion to the adult type of metabolism is compressed into the short space of about 15 days, facilitates the demonstration of the enzymatic changes that occur during this period of development.

The present study was carried out on both liver and brain, although the authors are aware of the limited interpretation to be placed upon the results with liver, owing to its hematopoietic function in the embryonic stage (2). The enzymes studied were succinic dehydrogenase and cytochrome oxidase as representative aerobic enzymes, and ATP-ase as a representative of the energy-depleting type of enzyme.

EXPERIMENTAL

Embryonic and newborn rats.—The rats were of the Sprague-Dawley strain, and the ages given for both the newborn rats and the embryonic rats are accurate to within 12 hours. The ages of the embryo rats were obtained from the time of conception, which was correct to within 12 hours on the basis of brief exposure to a male during estrus and the presence of spermatozoa in a vaginal smear taken within 12 hours from the time

* This work was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

† The data included in this paper were presented at the Gibson Island Conference on Growth, August 2, 1943.

the male was placed in the cage. Newborn rats were used one or two at a time from any given litter, so that each litter provided data at several different points on the time curve between 1 and 25 days. Data for adult rats were taken from the results of previous assays for the respective enzymes (4, 13), which were carried out in this laboratory at about the same time.

Enzyme methods.—Succinic dehydrogenase was assayed by the homogenate technic described by Schneider and Potter (13), in which the measurement is based on the oxygen uptake of the succinoxidase system with conditions such that succinic dehydrogenase is the limiting factor. Cytochrome oxidase was determined by using ascorbate as the substrate, as

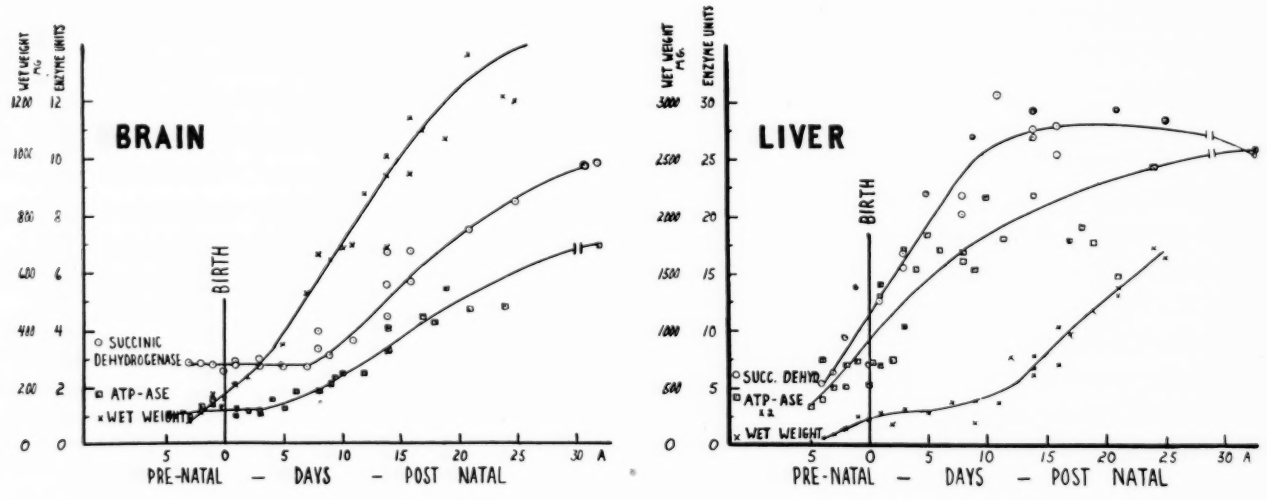


FIG. 1.—Increase in concentration of succinic dehydrogenase and ATP-ase in brain and liver of albino rats during late embryonic and neonatal life, compared with adult values. Enzyme concentrations expressed as units per mgm. fresh tissue.

previously described (13). The ATP-ase assay was that of DuBois and Potter (4). The methods are applicable to amounts of fresh tissue as small as 20 mgm., and are thus well suited for studies on embryonic and newborn rats.

RESULTS

The data for both brain and liver are presented in Fig. 1, in which are given the values for the total wet weight of the respective organs to show the extent of growth during the period from 5 days before birth to 25 days after birth. The time of birth, or zero on the abscissa, corresponds to an embryo age of 22 days. The values for the enzymes are given in terms of enzyme units. In the case of succinic dehydrogenase the data are expressed as the microliters of oxygen per mgm. of fresh tissue per hour. In the case of ATP-ase the data represent the micrograms of phosphorus split from ATP per mgm. of fresh tissue in 15 minutes for brain, while in the case of liver the actual values were multiplied by a factor of 2 for the purpose of graphing the results. Thus the value for ATP-ase in adult brain is 7.0 units, and for adult liver 12.9 units.

Fig. 1 shows that the concentration of both enzymes increased greatly during the 25 day period, and that by the end of this time the adult value had been reached. The conclusion that the increased enzyme concentration is not an artefact seems justified from the following considerations. According to Koch (7) the per cent dry weight of rat brains at birth was 10.42 and for adults 21.9, while Donaldson and Hatai (3) gave figures of 12.5 per cent at birth, 11.9 per cent at 4 to 8 days, 19.8 per cent at 25 days, and 21.5 to 22.5 per cent in the adult. Thus the threefold increase in succinic dehydrogenase in rat brain from birth to 30 days of age cannot be simply an expression of the increased dry weight. Similar considerations apply to ATP-ase, in which the increase was more than sixfold. Koch found that adult rat brains contained, in comparison with brains at birth, about the same amount of total sulfur, but about twice as much protein S, and 5 times as much lipoid S. With respect to protein, adult brains contained nearly twice as much on the fresh basis as did the brains at birth (7). Thus the increased concentration of succinic dehydrogenase and ATP-ase during this period is not a result of the increase in either solids or protein, and it follows that during this period the quantity of some proteins must have decreased while the quantity of the enzymes studied increased, and that the balance between the various types of protein must have been altered. The values for cytochrome oxidase are not shown in Fig. 1, since the values in general paralleled the succinic dehydrogenase values throughout the period studied.

Of considerable interest is the relation between sur-

vival time under anoxic conditions and the enzyme concentrations shown in Fig. 1. Both succinic dehydrogenase and ATP-ase in brain remain at the same concentration for the last few days *in utero* and during the first 5 or 6 days of extrauterine life, and begin to rise between the seventh and 15th days. During the first period the anoxic survival time falls rapidly and reaches the adult level at about the 11th day. The anoxic survival time is thus more closely correlated with the oxygen uptake of minced fresh tissue and with the succinic dehydrogenase and ATP-ase concentrations than it is with the Q_{O_2} values based on the dry weight of brain slices (6), suggesting that the *molarity* of a given enzyme in a tissue may be of greater significance than its concentration based on some other point of reference such as dry weight or protein phosphorus.

The findings in the case of liver present an interesting contrast to the brain data. In the case of brain, growth began at birth and the enzymes studied remained at embryonic concentrations for 5 or 6 days and then increased until the age of about 30 days. In liver this situation was reversed; the concentration of the enzymes rose rapidly during late uterine life, and continued after birth for about 15 days in the case of succinic dehydrogenase and about 30 days in the case of ATP-ase. Growth was slight in the period from birth to 10 days. Measurements on liver slices during this period of development show (2) that the anaerobic glycolysis decreases by one-half, while aerobic glycolysis increases and the Q_{O_2} remains approximately constant.

DISCUSSION

The results of this investigation show that there are notable increases in the concentration of the succinoxidase system in the brain and liver of the newborn rat during the period of greatest development, and that there are parallel increases in adenosinetriphosphatase.

Analyses for other biocatalysts in the tissues of newborn rats support the results of this investigation with respect to the increase of particular enzyme components. Thus Bernheim and Felsovanyi (1) determined the concentration of V-factor (coenzyme I+coenzyme II) in liver and kidney of embryos and newborn rats and found that the concentration in γ per gram wet weight increased from 100 in 2 week embryos to 150 at birth, and to the adult level of 500 to 600 by 7 to 9 days. Tyler (14) studied the effect of malonate on the respiration of brain tissue from newborn rats and found that at birth the rat is relatively insensitive to malonate, but that the sensitivity of adult brain is reached by the tenth day of life.

It appears that differentiation and specialization are associated with the development of an increased capacity for energy mobilization. This involves an increase in biocatalysts that are necessary for the combustion of fuel, such as the succinoxidase system, as well as an increase in ATP-ase. The results support the idea that ATP-ase is somehow related to function in brain and liver, but do not prove this point. The results cannot be said to test adequately the theory that growth is inversely correlated with the concentration of aerobic enzymes, for while the data show that the concentration of aerobic enzymes is high in the fully differentiated nongrowing tissue and low in the less differentiated growing tissues of the newborn rat, the issue is confused during the transition period by the fact that function is being superimposed on growth during this period. The data probably have a greater bearing on the problem of differentiation than on growth *per se,* but complete differentiation in a cell is generally associated with a loss in reproductive capacity. According to the theory, growth is regulated not by the absolute concentration of any particular enzyme, but rather by the balance that results from the effective concentrations of the enzymes that esterify phosphate and those that break down the phosphate esters. At this point we can only point out that representatives of both types of enzyme were increasing during the period studied. It is also considered significant that the concentration of succinic dehydrogenase in various experimental tumors (12) is almost identical with that of the brain at birth. The newborn liver had a concentration of succinic dehydrogenase intermediate between hepatomas and adult liver, but it has a functional load that is probably greater than that of brain at this period.

SUMMARY

1. Brain and liver from late embryonic and newborn rats up to the 30th day of postnatal life were compared with adult tissues in respect to their content of succinic dehydrogenase, cytochrome oxidase, and adenosine triphosphatase.

2. In brain the enzymes studied remained constant from about 3 days before birth until about 6 days after birth, and then increased rapidly so that the adult level was reached by the 30th day of life. The wet weight of the brains increased steadily throughout this period.

3. In liver the enzymes studied increased rapidly during late embryonic and early postnatal life, and the adult level was approximated within 10 to 15 days. Up to this time there was very little increase in the wet weight of the liver, but at this point its weight began to increase rapidly.

4. The results show that profound changes occur

in the enzymatic makeup of the newborn rat during a relatively short time after birth. The enzymes studied increased from 300 to 600 per cent during this period.

5. The enzymes studied represented both the energy-yielding type (respiratory enzymes) and the energy-depleting type (ATP-ase). The studies show that during the period of increasing functional load and increased differentiation both types of enzyme increased, that is to say, the potential rate of energy mobilization was increased.

6. The results are discussed in relation to the theory of cancer put forth by Potter, according to which growth is considered to be governed by the effective balance between the classes of enzymes mentioned above.

REFERENCES

1. Bernheim, F., and Felsovanyi, A. V. Coenzyme Concentration of Tissues. Science, **91**:76, 1940.
2. Burk, D. On the Specificity of Glycolysis in Malignant Liver Tumors as Compared with Homologous Adult or Growing Liver Tissues. A Symposium on Respiratory Enzymes. Madison: University of Wisconsin Press. 1942, pp. 235-245.
3. Donaldson, H. H., and Hatai, S. On the Weight of the Parts of the Brain and on the Percentage of Water in Them According to Brain Weight and to Age, in Albino and in Wild Norway Rats. J. Comp. Neurol., **53**:263-307. 1931.
4. DuBois, K. P., and Potter, V. R. The Assay of Animal Tissues for Respiratory Enzymes. III. Adenosinetriphosphatase. J. Biol. Chem., **150**:185-195. 1943.
5. Fazekas, J. F., Alexander, F. A. D., and Himwich, H. E. Tolerance of the Newborn to Anoxia. Am. J. Physiol., **134**:281-287. 1941.
6. Himwich, H. E., Baker, Z., and Fazekas, J. F. The Respiratory Metabolism of Infant Brain. Am. J. Physiol., **125**:601-606. 1939.
7. Koch, Matilde L. Contributions to the Chemical Differentiation of the Central Nervous System. I. A Comparison of the Brain of the Albino Rat at Birth with That of the Fetal Pig. J. Biol. Chem., **14**:267-279. 1913.
8. Okamoto, Y. Über Anaerobiose von Tumorgewebe. Biochem. Ztschr. **160**:52-65. 1925.
9. Negelein, E. Über die glykolytische Wirkung des embryonalen Gewebes. Biochem. Ztschr. **165**:122-133. 1925.
10. Potter, V. R. Biocatalysts in Cancer Tissue. IV. An Enzyme-Virus Theory Regarding Carcinogenesis. Cancer Research, **3**:358-361. 1943.
11. Potter, V. R. Biological Energy Transformations and the Cancer Problem. In Nord, F. F., and Werkman, C. H. Advances in Enzymology, Vol. IV. New York: Interscience Publishers, 1944, pp. 201-256.
12. Schneider, W. C., and Potter, V. R. Biocatalysts in Cancer Tissue. III. Succinic Dehydrogenase and Cytochrome Oxidase. Cancer Research, **3**:353-357. 1943.
13. Schneider, W. C., Potter, V. R. The Assay of Animal Tissues for Respiratory Enzymes. II. Succinic Dehydrogenase and Cytochrome Oxidase. J. Biol. Chem., **149**:217-227. 1943.
14. Tyler, D. B. Effect of Malonate and Iodoacetate on Respiration of Brains of Rats of Various Ages. Proc. Soc. Exper. Biol. & Med., **49**:537-539. 1942.

Immunological Factors that Influence the Neoplastic Effects of the Rabbit Fibroma Virus*

F. Duran-Reynals, M.D.

(From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication June 9, 1944)

It is known that the virus of the rabbit fibroma (41), injected into the skin of adult, domestic rabbits, induces a mild, localized, neoplastic lesion that invariably regresses after attaining its maximum size at from 8 to 12 days after inoculation. Tumor regression is followed by a solid immunity. The virus injected into the blood stream or peritoneum is ineffective.

In a previous publication (14) it was shown that the same virus, as found in fresh tumors, injected in large amounts into the skin and veins of newborn rabbits, induced local and generalized lesions of a nature essentially inflammatory and caused death in from 4 to 7 days. However, if the virus after storage in glycerol was injected into these hosts local tumors developed, which grew steadily for several weeks and then either regressed or killed the host. In the latter instance the picture was one of typical malignancy with a large, firm tumor surrounded by satellite and sometimes with widespread growths.

What was learned concerning the reactions following the injection of viruses of avian tumors into newborn birds (12, 13, 15, 32) suggested that factors concerned with the immunological maturation of the host might have been back of these comparable phenomena in rabbits. The present paper deals with the experimental approach to this view.

MATERIALS AND METHODS

Albino rabbits of a single breed were used throughout. The fibroma was kept by passage through adult individuals, using the skin growths as source material. The tumor tissue was extracted with saline solution in varying proportions, either immediately after removal from rabbits or after having been stored in 50 per cent glycerol in saline solution at 2° to 4° C. for variable periods.

The newborn rabbits were injected intracutaneously and subcutaneously in the groin. Immediate mortality was high, generally because the baby rabbits were killed by their mothers.

After death the primary tumor and the lung, liver, spleen, and kidney were routinely examined for both microscopic lesions and for virus content. For the microscopic studies hematoxylin-eosin and Masson stains were used after fixation in 10 per cent formalin. For the detection of virus the tissue was ground with sand and extracted with 5 volumes of physiological saline, and 1 cc. of the extract was injected intradermally into adult rabbits. The results were read 8 or 10 days later.

Since from previous experience we knew that there is a fairly good relationship between the size of tumors induced by an infectious extract and the amount of virus present in it, as determined by its end-titer, we have graded the virus content of tissues from +, indicating tumors of from 1 to 2 sq. cm., to +++++, indicating tumors of from 10 to 18 sq. cm. The symbol ± indicates a tumor smaller than 1 sq. cm. or a doubtful tumor.

Titration of virus-neutralizing antibodies was carried out by mixing equal parts of serum, either undiluted or in dilutions of 1:10, 1:50, 1:100, 1:500, and 1:1,000, with an extract, diluted 1:100, of fresh fibroma tissue. After a contact of 60 min. at 37°, 0.5 cc. of each mixture was injected intradermally in one flank of adult rabbits. As a control the virus preparation employed was each time titrated on the other flank of the same rabbit, by injecting 0.25 cc. of each dilution mixed with 0.25 cc. of saline. It was not unusual to find that fibroma extracts diluted as high as 1:10,000,000 still induced tumors. The results of the virus neutralization were appraised by differences in the size of the tumors elicited by serum-virus mixtures and by the 1:100 control dilution respectively. These differences were expressed by + signs as follows: +++++ indicates total neutralization; ++++ pronounced; +++, medium; ++, weak; and + very weak neutralization. Absence of neutralization is indicated by a − sign.

One may well state here that several normal rabbits were studied for the presence of natural antibodies against the fibroma virus. Total suppression of the tumor by undiluted serum was sometimes ob-

* This investigation was aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

tained, but in these cases the sera diluted 1:10 exerted practically no effect.

Generalized spread of the virus and immune responses in newborn and adult rabbits.—As a preliminary step we studied a group of adult rabbits and another group of newborn rabbits bearing fibromas, from the following points of view: (a) presence of the virus in blood and viscera, and (b) degree of antibody response against the virus. A previous investigation by Hurst (27) had shown that virus may be found in the viscera of adult rabbits from the fifth to the eighth day after infection.

Nine adult rabbits were injected intradermally in several areas, each with 1 cc. of extracts of fresh fibroma tissue diluted 1:2. The areas of the tumors that resulted measured from 10 to 50 sq. cm. Another set of rabbits 2 or 3 days old and belonging to 2 litters were injected, each under the skin of the groin, with 1 cc. of a 1:5 dilution of fibroma tissue that had been stored for 2 days in saline-glycerol; hence its activity was somewhat reduced. Tumors measuring from 10 to 30 sq. cm. developed in these young rabbits, which proved fatal in all but one of them. The adult rabbits were killed from 5 to 15 days after injection. By cardiac puncture samples of blood were collected from the adult rabbits at death, and from the young rabbits 7 to 9 days after inoculation, more blood being later secured from those that survived longer. The blood was drawn into a syringe and before it clotted 0.5 cc. was injected into the skin of rabbits; the rest was used for the testing of antibodies. The presence of virus in the viscera was investigated in the manner previously described. The results of these tests are expressed in Table I.

It is clear from Table I that in adult rabbits virus was never detected in the blood, and was detectable in the viscera only up to the eighth day after inoculation of the virus. On the other hand, viral antibodies were found in the blood at the fifth day after inoculation, and judging by their titer they were present there before that time.

On the contrary, in the rabbits injected 2 and 3 days after birth virus was detected in blood up to the 13th day, and in the viscera up to the 21st day after infection; no antibodies were detected until at least 9 days after infection, and in the few cases studied the antibody response after 9 and 15 days was weaker than in comparable sera from adult rabbits.

Postmortem examination showed the virtual absence of gross and microscopic lesions in adult rabbits. Lesions in the young rabbits, including the tumors, will be described later.

Immunity to reinfection in adult and young rabbits bearing tumors.—As a corollary of the study described above the following experiments were carried out.

TABLE I: VIRUS GENERALIZATION AND IMMUNE REACTIONS IN ADULT AND NEWBORN RABBITS

No. of rabbit	Age	Time of death, days	Distribution of virus in tissues						Neutralization of virus by serum dilution				Time after injection when blood was collected
			Tumor	Lung	Liver	Spleen	Kidney	Blood	Und.	1:10	1:50	1:100	1:500	1:1000	
1	4 mo.	5	+ + + + + +	−	+ + +	+ ǂ	+	−	+ +	+ +	+ +	+ +	+ +	−	at death
2	"	7	+ + + + + +	+	+				+ +	+ +	+ +	+ +	+ +	+	"
3	"	8	+ + + + + +	+	+ +		+		+ +	+ +	+ +	+	+	+	"
4	"	8	+ + + + + +	−	+		+		+ +	+ +	+ +	+ +	+ +	+	"
5	"	12	+ + + + + +	−	+	−	+	−	+ +	+ +	+ +	+ +	+ +	−	"
6	"	12	+ + + + + +	−	+	−	+	−	+ +	+ +	+ +	+ +	+ +	+ +	"
7	"	13	+ + + + + +	−	+	−	+	−	+ +	+ +	+ +	+ +	+ +	+ +	"
8	"	15	+ + + + + +	−	+	−	+	−	+ +	+ +	+ +	+ +	+ +	+ +	"
9	"	15	+ + + + + +	−	+	−	+	−	+ +	+ +	+ +	+ +	+ +	+ +	"
10	3 days	8	+ + + + + +	+	+ +	+	+	+	−	−	−	−	−	−	7 days
11	"	8	+ + + + + +	+ ǂ	+	+	+	+	−	−	−	−	−	−	8 "
12	"	11							−	−	−	−	−	−	9 "
13	"	11							−	−	−	−	−	−	9 "
14	"	12	+ + + + + +	+	+	+	+	+	−	−	−	−	−	−	9 "
15	"	12	+ + + + + +	−	+	−	+	+	−	−	−	−	−	−	9 "
16	"	13	+ + + + + +	+	+	+	+	+	−	−	−	−	−	−	9 "
17	2 days	13	+ + + + + +	+	+	+	+	−	−	−	−	−	−	−	9 "
18	"	15	+ + + + + +	+	+	+	+	−	−	−	−	−	−	−	9 "
19	"	21	+ + + + + +	+ ǂ	+	+	+	−	−	−	−	−	−	−	9 "
Same									+	+	+	+	+	−	16 "
20	"	30 killed*							−	−	+	+	−	−	9 "
Same	"								+ +	+ +	+ +	+ +	+ +	−	16 "
Same	"								+ +	+ +	+ +	+ +	+ +	+	23 "

* Tumor regressed.

Fibroma tissue from an adult rabbit was desiccated and stored at 2° to 4° C. so as to have a virus preparation of uniform activity. On 6 successive days 0.25 gm. of the desiccate was extracted with 10 cc. of saline, and 1 cc. of this extract was injected intradermally into each of 2 areas on the depilated flanks of 4 rabbits. The experiment was terminated 10 days after the injections, with the following results. The tumors resulting from the first set of injections measured on the average 10 sq. cm.; those from the injections 24 hours later measured 8 sq. cm.; and the size progressively decreased until the fifth day, when no tumors resulted from the injections. That the activity of the preparation had not been impaired by desiccation was shown by appropriate injections into control rabbits.

Since it was not possible to duplicate this experiment exactly in newborn rabbits, the following procedure was substituted. Six rabbits, 5 hours old (Nos. 14 to 19 of Table IV), were injected in the right groin with 1 cc. of minced tissue from fibromas that had been kept for 4 months in glycerol-saline. Twelve days later, when the tumors from the first inoculation were barely noticeable, the rabbits were injected in the left groin with 0.5 cc. of minced tumors that had been kept 7 days in glycerol-saline. This preparation was rather active, as injection into other rabbits showed. The results were as follows. All injections induced tumors, but those resulting from the first attained a size of 10 or more sq. cm., while those from the second injections attained at best a size of 4 mm. in diameter. It is clear that some immunity to reinfection had established itself in the young rabbits 12 days after the first infection, but this immunity certainly could not compare with that shown by the adult rabbits.

In other experiments, involving rabbits infected shortly after birth in which tumors had regressed, or no tumors had been found, tests were made at the age of 2 months with active virus. No tumors ever arose, showing that with advancing age these animals had developed a complete immunity.

Attempts to overcome the resistance of adult rabbits by increasing the amount and virulence of the virus.—The experiments consisted of 16 weekly passages of the fibroma through the rabbit by means of tumor extracts at 1:6 dilution mixed with bull-testicle extract at 1:2 dilution (spreading factor). One cc. of this mixture was injected intradermally and the injections were repeated 4 times, so that the flanks of the rabbits were practically covered with tumors. Moreover, large amounts of testicle extract were injected daily into the blood stream. The increase of virulence of the virus was manifest from the fourth passage on. Definite lesions, inflammatory in the gross, were noticeable 1 day after inoculation; tumors were definitely formed after another day, and 3 days later they had attained sizes of 40 sq. cm. and were surrounded by considerable edema.

Despite all these measures the evolution of the tumors was the same. No generalization of the fibromas was ever obtained and no virus was detected in the blood. Intraperitoneal and intravenous injections of the virus from the enhanced lesions failed to induce lesions in rabbits.

The effect of variable amounts of fresh virus on rabbits from 1 to 15 days of age.—Two experiments were carried out on 6 litters of rabbits. In the first experiment the amount of virus injected was, roughly, directly proportional to the age of the rabbits. In the second, the amount of virus was rather arbitrary. A different virus preparation was used in each experiment. Blood for investigation of antibodies was secured at death or a few days before. The results of these 2 experiments are summarized in Tables II and III respectively.

The most important conclusion from these experiments is that small amounts of fresh virus, injected into rabbits from 1 to 15 days of age, produced the same effects as larger amounts of glycerol-stored virus. In almost every case tumors were induced that grew to a size of sometimes 25 or more sq. cm., generated many satellite growths, and in one case produced widespread tumors. Growth was either progressive until death or was followed by regression, according to factors depending on the amount of virus and the age of the host. Other aspects of the results will be analyzed later.

The effect of varying amounts of glycerol-stored virus on rabbits from 6 hours to 9 days of age.—With the view of determining the optimal conditions, as regards the amount of stored virus injected and the age of the rabbits, that lead to the production of a generalized neoplastic disease a total of 31 rabbits from 6 hours to 9 days of age and belonging to 6 litters was injected with either 1 or 3 cc. of 1:5 or 1:20 extracts of fibroma tissue that had been stored 3 days in glycerol, the amount of virus being roughly proportional to the age of the host. The results added little to what was already known. All animals developed tumors, of which 22 regressed, often after attaining a large size, while those that grew progressively until the death of the host never induced widespread growths. A point of interest is that tumors often either grew progressively or regressed in litter mates identically injected.

Two more experiments were carried out by injecting 19 rabbits, aged 5, 6, and 24 hours, with 1cc. of minced fibroma tissue that had been stored 4 months in glycerol. The results are given in Table IV. Tumors appeared in all but 3 cases, and these tumors regressed in 11 rabbits and grew progressively in

* Tumor regressed.

TABLE II: VIRUS GENERALIZATION AND IMMUNE REACTIONS IN YOUNG RABBITS INJECTED WITH FRESH VIRUS

No. of rabbit	Age, days	Virus dilution injected, 1 cc.	Evolution of tumor	Time of death, days	Distribution of virus in tissues					Neutralization of virus by serum dilution					
					Tumor	Lung	Liver	Spleen	Kidney	Und.	1:10	1:50	1:100	1:500	1:1000
1	15	1:20	Regr.												
2	"	"	"												
3	"	"	No growth							+++++	+++++	++++	++++	++++	+++*
4	"	"	Regr.												
5	"	"	No growth							+++++	+++++	++++	++++	++++	+++*
6	8	1:200	P. G.	12	++	+++++	++	+	+	+++++	++++	+++	++	++	−
7	"	"	"	13											
8	"	"	"	14	+++++	++	−	−	−						
9	"	"	"	14	+++++	++	+	−	+						
10	"	"	"	14	+++++	++	−	−	−	+++++	++++	++++	+++	+++	++
11	"	"	"	14	+++++	−	−	−	−	+++++	++++	++++	+++	+++	++
12	6	1:2000	"	25	+	−	−	−	−	+++++	+++++	+++++	+++++	++++	++++
13	"	"	"	26	+++++	−	−	−	−	+++++	++++	++++	++++	++++	++++
14	"	"	"	29	+	−	−	−	−						
15	"	"	"	30	+	−	−	−	−	+++++	++++	++++	++++	++++	++++
16	3	1:20,000	Regr.												
17	"	"	"												
18	"	"	"												
19	"	"	"							+++++	+++++	+++++	+++++	++++	++++*
20	"	"	"												

P. G.—Progressive growth.
Regr.—Initial growth followed by regression.
* Blood for neutralization tests secured 40 days after virus inoculation.

TABLE III: VIRUS GENERALIZATION, WIDESPREAD GROWTHS, AND IMMUNE REACTIONS IN YOUNG RABBITS INJECTED WITH FRESH VIRUS

No. of rabbit	Age, days	Virus dilution injected, 1 cc.	Evolution of tumor	Time of death, days	Distribution of virus in viscera					Neutralization of virus by serum dilutions						Widespread growths
					Tumor	Lung	Liver	Spleen	Kidney	Und.	1:10	1:50	1:100	1:500	1:1000	
1	1	1:2000	P. G.	14	+++++	+++++	+++++	++	+++++	+++	+	+	−	−	−	
2	"	"	"	25	−	−	−	−	−	+++++	+++++	+++	++	+	−	Fibromas in thymus and spleen.
3	5	1:10	"	17	+++++	−	−	−	−	+++++	+++++	+++++	+++++	++++	++	
4	"	"	"	18	+++++	±	++	+	++	+++++	+++++	+	+	+	−	
5	"	"	"	18						+++++	+++++	+++++	+++++	+++++	++++	
6	"	"	"	10	+++++	−	−	−	−	+++++	++++	++++	++++	+++	+++	
7	"	"	"	13												
8	"	"	"	20	+++++	−	−	−	−	+++++	+++++	++++	++++	++++	++++	
9	"	"	"	20	+++++	−	−	−	−	+++++	++++	++++	++++	++++	++++	

P. G.—Progressive growth.

TABLE IV: VIRUS GENERALIZATION, WIDESPREAD GROWTHS, AND IMMUNE REACTIONS IN NEWBORN RABBITS INJECTED WITH GLYCEROL STORED VIRUS

No. of rabbit	Age, hrs.	Evolution of tumor	Time of death, days	Distribution of virus in viscera					Neutralization of virus by serum dilutions						Widespread growths
				Tumor	Lung	Liver	Spleen	Kidney	Und.	1:10	1:50	1:100	1:500	1:1000	
Exp. I															
1	6	P. G.	18	+++++	+++++	++++	++	+++++	+++++	+++++	+++++	+++	++	+	Fibromas in kidney and skin
2	"	"	25	+++++	−	−	−	−	+++++	+++++	+++++	+++++	++++	++++	
3	"	"	25	+++++	+++	++	++	+++							Fibromas: liver, lung, and kidney
4	"	Regr.							+++++	+++++	+++++	+++++	++++	++++*	
5	"	"							+++++	+++++	+++++	++++	++++	++++*	
6	"	"							+++++	++++	++++	++++	++++	+++*	
7	24	P. G.	14												
8	"	No tumor													
9	"	"													
10	"	Regr.													
11	"	No tumor													
12	"	Regr.													
13	"	"													
Exp. II															
14	5	Regr.			−	−	−	−							
15	"	P. G. Regressing at death	36	+++	−	−	−	−							Fibromas in ribs, ears, and kidneys
16	"	Regr.													
17	"	"													
18	"	"	57 killed	++	−	−	−	−							
19	"	"	39 killed	+++	−	−	−	−							

P. G.—Progressive growth.
Regr.—Initial growth followed by regression.
* Blood for neutralization tests secured 65 days after virus inoculation.

the rest. Widespread growths were found in 3 cases. Individual differences of susceptibility were also manifest. The glycerol-stored virus used in these experiments, tested on adult rabbits, produced skin lesions that were small; after 4 months' storage of the virus lesions were practically absent.

the tumors in both rabbits and in the viscera of the rabbit that died 18 days after injection, but absent from the viscera of the other. These experiments indicate the effectiveness of the virus in the peritoneal cavity of newborn rabbits.

Characteristics of the tumors induced by injection of virus into newborn rabbits.—In both their gross and microscopic features and their evolution the tumors were identical, whether they were induced by small amounts of fresh virus or by larger amounts of virus that had been stored in glycerol.

FIG. 1.—Inflammatory aspect of a fibroma induced by injection of a newborn rabbit with a dose of virus that killed the animal in 8 days. Natural size.

FIG. 2.—Type of tumor induced by injection of the newborn rabbit with small amount of virus. The animal died 3 weeks after injection. Natural size.

The effect of glycerol-stored virus injected into the peritoneal cavity of newborn rabbits.—Two rabbits, 24 hours old, were injected intraperitoneally with 1 cc. of minced tissue from fibromas that had been stored 4 months in glycerol. The rabbits died, 18 and 34 days after inoculation, with large primary tumors in the abdominal cavity and several secondary nodules attached to abdominal viscera. Virus was plentiful in

If the virus, despite the dilution of the extract, was still too active or the storage in glycerol was too short the disease, especially in particularly susceptible

hosts, showed a few traits in common with the inflammatory condition that results when large amounts of fresh virus are injected. The lesions were then noticeable a few days after infection, the skin covering the tumors and regional lymph nodes was hemorrhagic and edematous, and the tumors themselves were soft and very viscid (Fig. 1). The viscera were often congested, and death took place within the first 12 days. Still, it is clear from the pictures that the neoplastic features were by far predominant. Microscopically, some areas of the tumors showed the loose, inflammatory aspects shown in Fig. 3, but in many other areas the growths were just as compact as older tumors.

If, because of dilution of the fresh extract or the length of storage in glycerol, the amount of virus injected was really small, tumors were not clearly noticeable until from 7 to 10 days after infection. By that time, and in later stages as well, they were more firm, well demarcated, free of inflammatory signs, and, unless about to regress, free of necrosis (Fig. 2). Microscopically, the growths were compact in general and had the features shown in Fig. 4. Mitoses were rare.

In all cases growth was rapid, and tumors of 25 and 30 sq. cm. sometimes developed within the first 2 weeks, that is, to sizes far above those of comparable tumors in adult rabbits. Then they remained stationary until the host died or until regression of the growths took place. Development of satellite nodules in the skin was a common occurrence, and some were observed in the diaphragm. Vicinal lymph nodes were often swollen, and sometimes involved by extension of the growth.

In the present investigation widespread growths were observed in 4 cases, the same number observed in the 1939 experiments. In Table V is listed the incidence of these growths according to the age of the rabbit at the time of injection, and it is seen that 7 of the 8 cases occurred in the 39 rabbits injected with virus within the first 24 hours after birth.

Widespread growths were observed in the thymus, liver, kidney, lung, spleen, ribs, and skin of the ears and, in the 1939 series, in the lymph nodes, also. As shown in Figs. 5, 6, and 7 they appeared in the form of distinct, firm, white, well-circumscribed masses from 1 to 7 mm. in diameter, while others were detected only after microscopic examination. Microscop-

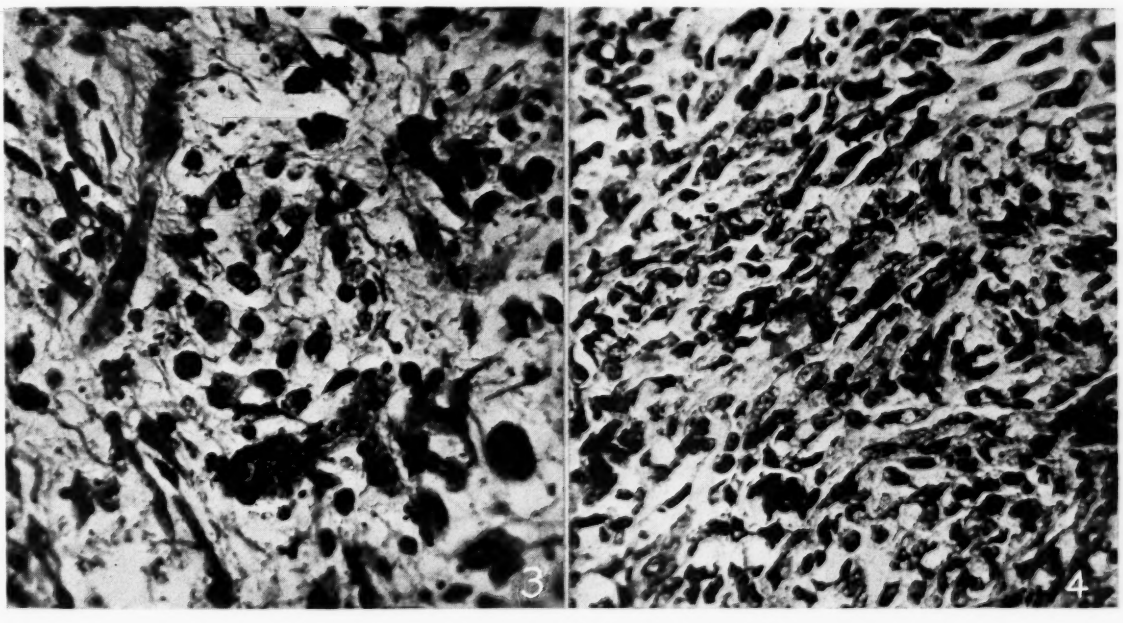


FIG. 3.—Tumor of rabbit of Fig. 1. Mag. × 325.
FIG. 4.—Tumor of rabbit of Fig. 2. Mag. × 300.

TABLE V: INCIDENCE OF WIDESPREAD GROWTHS ACCORDING TO AGE OF INJECTED RABBITS

No. of rabbits	Age	No. showing widespread growths	
19	5 hours	3	From present experiments
9	1 day	1	
4	2 days	0	
12	3 days	0	
7	5 days	0	
13	6 days	0	
6	8 days	0	
15	9 days	0	
5	15 days	0	
11	1 day	3	From 1939 experiments
3	3 days	1	
4	4 days	0	
2	5 days	0	
Total 100		8	

ically they could sometimes be seen arising from the adventitia of blood vessels (Fig. 8), thus corroborating previous observations (14). In the ribs they apparently arose from the periosteum (Fig. 9), and invaded the marrow spaces (Fig. 10). In the skin of the ear (Fig. 11) a pronounced epithelial stimulation was noticeable, a reminder of the epitheliotropism of the virus. All these growths consisted of typical fibroma cells, as is well shown in Figs. 10, 11, and 12. Mitoses were more frequent than in the primary tumors. The lesions sometimes observed (Figs. 13 and 14), and suggesting occlusion of vessels by fibroma cells, may well have been the result of endothelial stimulation such as that described by Hurst (25) in myxomatosis and by Ahlström and Andrewes (2) in fibromatosis in specially prepared rabbits, but the possibility of their being the result of cell emboli must not be overlooked; see Fig. 11 of (14).

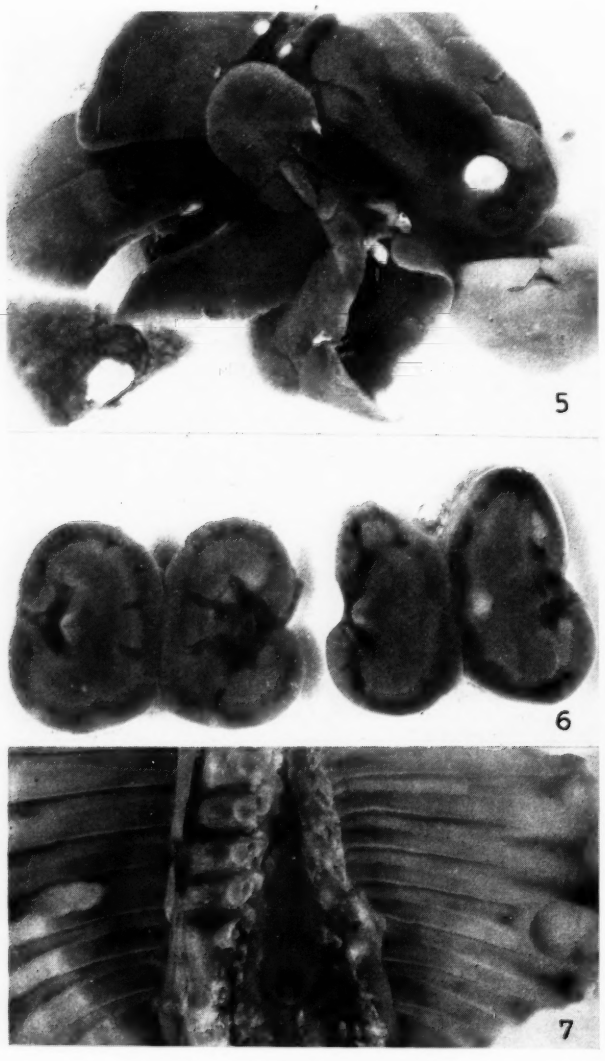


FIG. 5.—Visceral growths in rabbit No. 3 of Table IV. Injected with virus at the age of 6 hours. Died 25 days later.

FIGS. 6 and 7.—Kidney and rib growths in rabbit No. 15 of Table IV. Injected with virus at the age of 5 hours. Died 36 days later. Natural size.

We often came across structures suggesting the various inclusion bodies described by several authors (4, 17, 34, 37, 41, 47) in fibroma and myxoma cells, but no special effort was made to study them.

Regression of even large tumors occurred frequently, and where tests were made no virus was found in these receding tumors. In one case (rabbit No. 2 in Table III) the necrosis of peripheral cells and a fibrous reaction suggested that a tumor in the thymus was about to regress.

Often in rabbits that died of their tumors, and occasionally in those with widespread growths, lesions of a proliferative and degenerative type were observed in the viscera. These consisted of degeneration of the hepatic and renal parenchyma, patches of hemorraghic pneumonia, and lymphocytic depletion in the spleen with proliferation of the cells of the adventitia. We can well dispense with details concerning all these lesions since a full description of them has been given elsewhere (14).

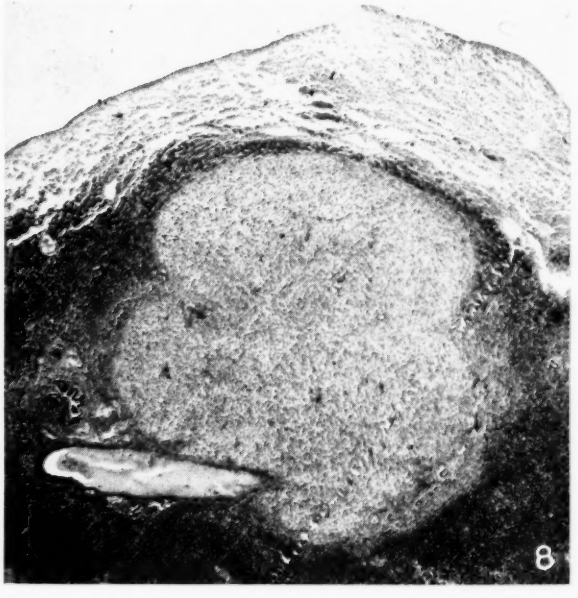
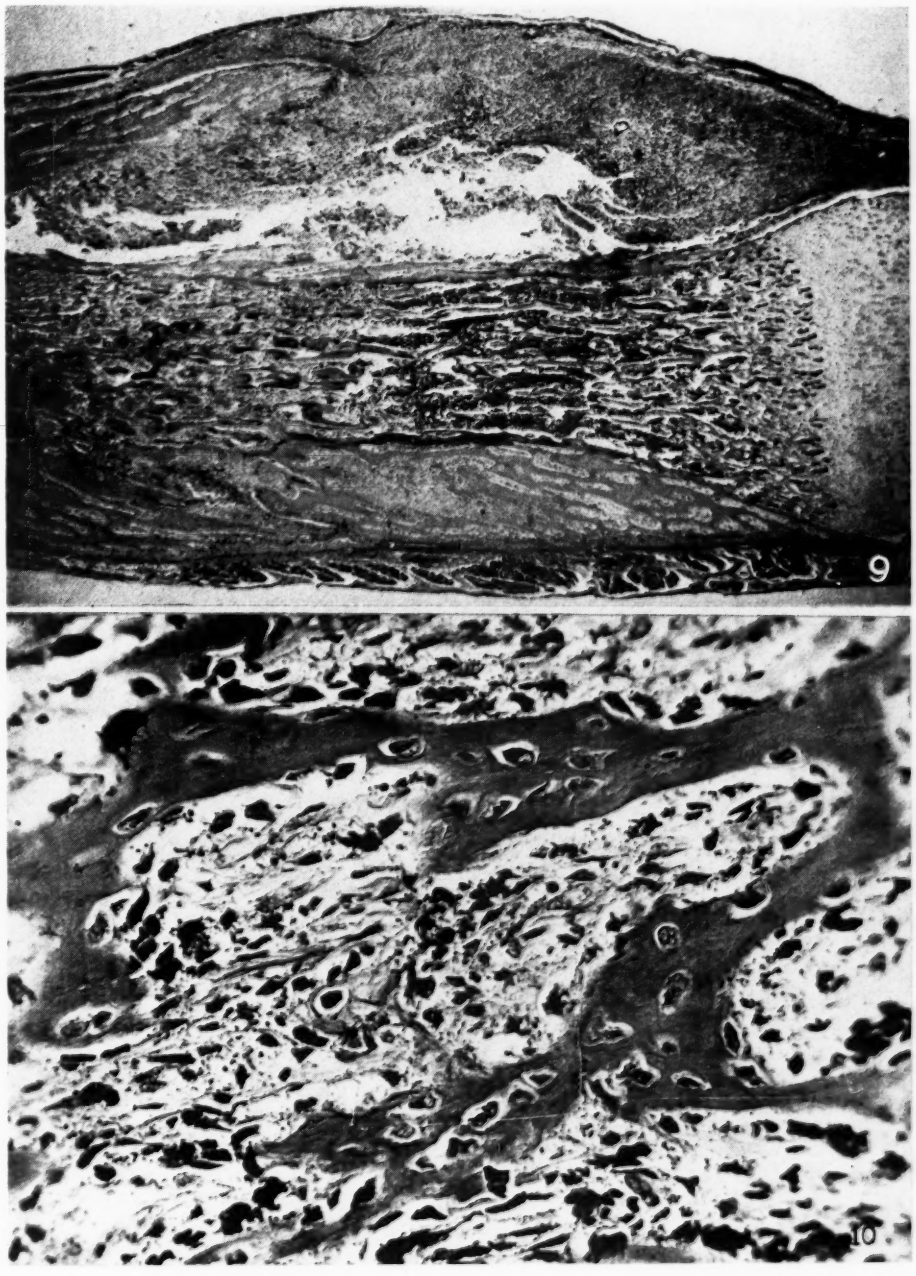


FIG. 8.—Liver fibroma arising from the adventitia of a vessel in rabbit of Fig. 5. Mag. × 12.

The tumors induced in adult rabbits injected intradermally with extracts of tumors or of viscera from infected newborn rabbits differed in no way from tumors elicited by the ordinary virus. They all attained a maximum size after 8 to 12 days, remained stationary for another few days, and finally regressed. Cell suspensions from the generalized fibromas were also injected into the skin and testes of adult rabbits. Again tumors of the ordinary sort resulted, those growing in the testes persisting a longer time.

DISCUSSION

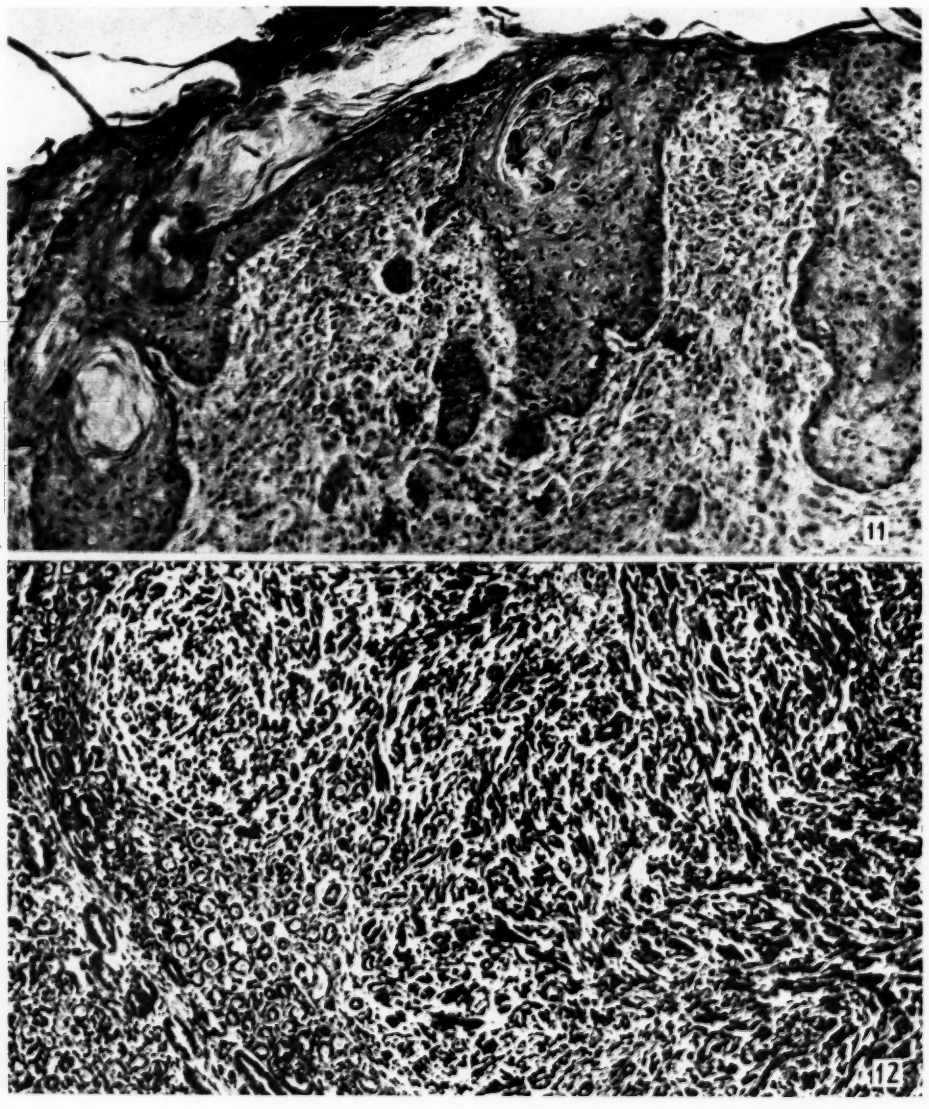
The important fact disclosed in the present and in a previous study (14) is that a single virus, that of the rabbit fibroma, is capable of inducing, on the one hand, an acute, predominantly inflammatory and destructive disease, and, on the other hand, a neoplastic disease of much longer duration showing traits of malignancy. In order to evaluate properly these findings one has to correlate them not only with what is known of the fibroma virus, but also with our knowledge of another highly pathogenic and epidemic variant of the fibroma, that of the rabbit myxoma. This has been done in Table VI, where it is seen that factors of fundamental importance in infection and resistance are the cause of a prodigality of manifestations by the virus, which is evidently endowed with a pronounced ability for variation and adaptation.



FIGS. 9 and 10.—Periosteal tumors in ribs of rabbit of Fig. 6. Mag. × 14 and × 325 respectively.

There is no doubt today that the agents of the rabbit myxoma of Sanarelli (39) and the rabbit fibroma of Shope (41) are but variants of the same virus. Their size is the same (38, 40); their species and tissue affinities are identical; there is a fundamental underlying similarity in the lesions induced (1, 25, 28, 39, 41, 42, 47); cross immunity effects be-

tween them have been demonstrated (7, 33, 35, 42-44); and, finally, the experimental transformation of the fibroma virus into several strains of myxoma virus, one of them nonlethal, has been accomplished (6, 8, 9, 19, 27). As adding further to the functional flexibility of the virus one must mention the following: (a) the spontaneous transformation of the ordinary, neoplastic fibroma variant into inflammatory and intermediate strains, as observed both in the United States and England; [1] (b) the induction of inflammatory, generalized, dermal lesions by both the neoplastic and inflammatory strains of the fibroma variant, as occurring in England but not in the United States, in several identical breeds of rabbits (3, 45); (c) the obtaining of new and sometimes nonlethal strains of the myxoma variant by passages of the virus through the brain of domestic rabbits (neuromyxoma, 26), and through chick embryos (21, 24); (d) the distinct differences in the disease induced by both variants in domestic rabbits (*Oryctolagus*) and in wild rabbits (*Sylvilagus*) (5, 7, 23, 41, 43); by the myxoma variant in the pregnant domestic rabbit (46) and in rabbits immune to fibroma (29, 31, 44).



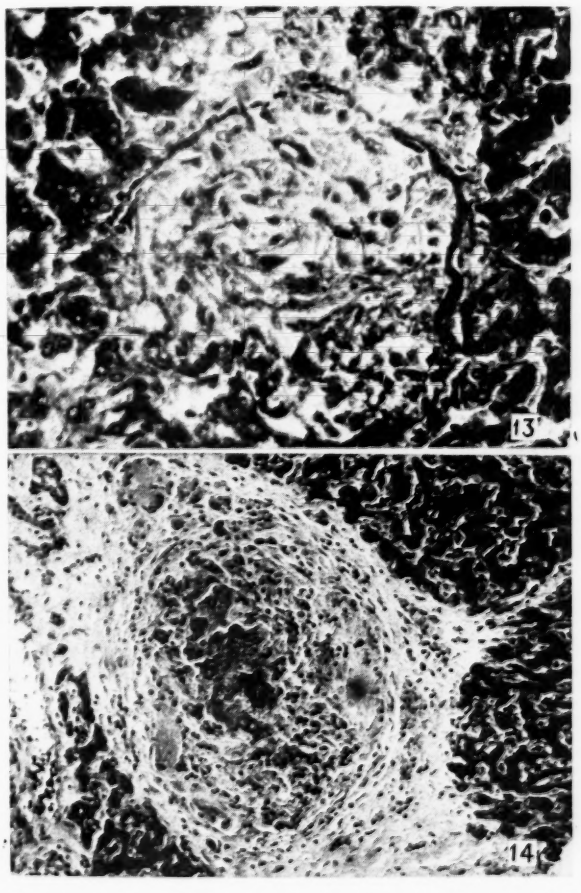
FIGS. 11 and 12.—Fibromas in the skin of ear and in the kidney of rabbit No. 1 of Table IV. Mag. × 85, and × 110 respectively.

When in our studies the fibroma variant was injected in large amounts into newborn rabbits an acute condition, lethal in a few days, resulted. It is interesting to point out the many analogies between this disease and that induced in adult domestic rabbits by the much more pathogenic myxoma variant. Besides the usual and very conspicuous signs of inflammation and a great abundance of a typical, mucoid, inter-

[1] The inflammatory strain, like the myxoma variant (18, 20, 21) has been cultivated in tissue cultures; the neoplastic strain has not.

cellular substance, one observes in both conditions proliferation of the same cells coexisting with destruction not only of preexisting tissues such as muscle, fat, and the parenchyma of several organs, but also of the newly formed mesenchymal cells. In both conditions a generalized spread of the virus until death, and susceptibility to any route of inoculation are the rule.

When the newborn rabbits were injected with the fibroma variant in small amounts, by using either glycerol-stored virus or small inocula of fresh tissue



FIGS. 13 and 14.—Lesions suggesting intravascular growths of possible endothelial origin in rabbit of Fig. 5. Mag. × 109 and × 217 respectively.

extracts, tumors resulted that grew for several weeks, generated satellite nodules and sometimes widespread growths, and often killed the host. Peritoneal injections were equally effective. Virus was present in blood and viscera during the first 9 days and in viscera for 2 weeks, a sharp contrast with adult rabbits, in which virus generalization lasts only a few days and never affects the blood.

It is important to correlate the progressive growth and generalization of the fibromas with the findings of Ahlström and Andrewes (2), who treated adult domestic rabbits with chemical carcinogens and then infected them with fibroma virus. The regression of intradermally induced tumors was much delayed, the growths behaving like those in the naturally occurring disease of the wild rabbit. Those induced by subcutaneous injections grew progressively, "coming to resemble true neoplasms," and intraperitoneal injections were sometimes effective. Intravenous inoculations of virus induced tumors at sites injected with the carcinogen, and in addition produced generalized fibromas closely resembling the generalized lesions observed by us, although it seems probable that the tumors were produced by the intravenously injected virus rather than by virus from the main tumor.

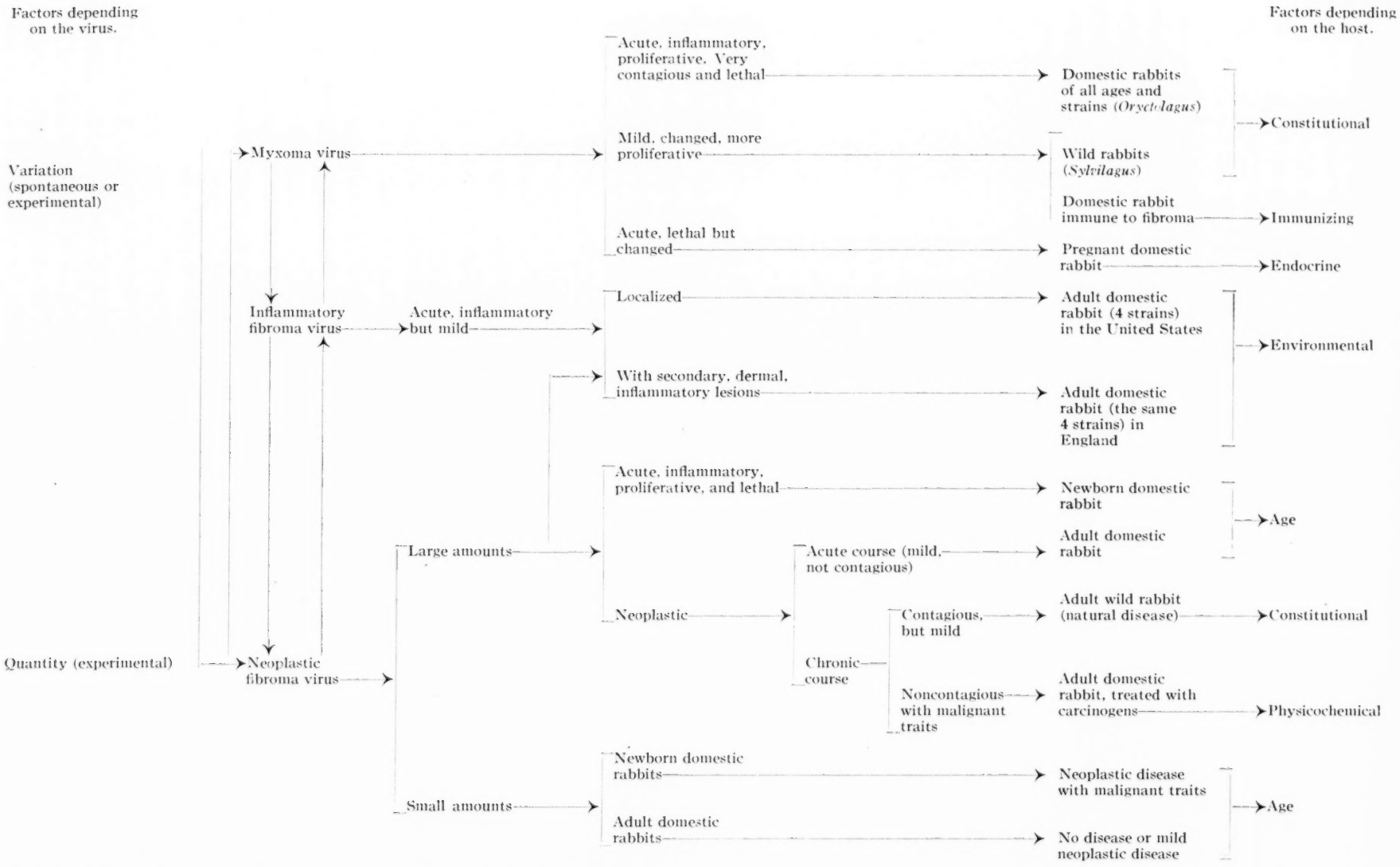
X-rays have been shown to exert the same effect (11), but noncarcinogenic compounds were found inactive (2). The generalized fibromas were observed by us to arise from the adventitia of vessels whose cells are stimulated in the disease induced in adult rabbits by the myxoma variant, and in the newborn by large amounts of the fibroma variant. Therefore, many of these lesions were in all probability late manifestations of an early infection of distant cells by the virus, but not because they arose in this way should they properly be considered as metastases, since in avian tumors comparable growths can be generated by either virus or cells (13). Moreover, the presence of intravascular growths in the animals showing widespread tumors may point to the fibroma cells carried by the blood stream as their occasional originators; see Fig. 11 (14).

What determines the different evolution of the disease in the newborn rabbit injected with either large or small amounts of virus? Since small amounts of fresh virus produced the same effects as larger amounts of glycerol-stored virus it would seem that storage in glycerol did nothing but reduce the amount of virus. Furthermore, extracts of either inflammatory or neoplastic lesions caused the usual mild fibromas in adult rabbits. It is obvious, therefore, that the virus did not vary, and the answer to the above question must be sought in alterations in the host.

It has been shown in the present study that adult domestic rabbits develop a remarkable resistance to reinfection, which is already noticeable 24 hours after inoculation, and the antibody response is prompt and vigorous. On the contrary, in rabbits injected within the first 24 hours after birth with glycerol-stored virus resistance to reinoculation was not yet complete 12 days after infection, and the antibody response was slow and poor. Therefore, the virus injected in large amounts into newborn rabbits finds a host entirely devoid of resistance and unable to elaborate antibodies for many days to come. A condition that is largely inflammatory results, and death ensues in a few days.

The evolution and fatal issue of the disease induced

TABLE VI


Factors depending on the virus.
Factors depending on the host.
Variation (spontaneous or experimental)
Quantity (experimental)
Myxoma virus
Inflammatory fibroma virus
Neoplastic fibroma virus
Acute, inflammatory but mild
Large amounts
Small amounts
Acute, inflammatory, proliferative. Very contagious and lethal
Mild, changed, more proliferative
Acute, lethal but changed
Localized
With secondary, dermal, inflammatory lesions
Acute, inflammatory, proliferative, and lethal
Neoplastic
Chronic course
Contagious, but mild
Noncontagious with malignant traits
Newborn domestic rabbits
Adult domestic rabbits
Domestic rabbits of all ages and strains (Oryctolagus)
Wild rabbits (Sylvilagus)
Domestic rabbit immune to fibroma
Pregnant domestic rabbit
Adult domestic rabbit (4 strains) in the United States
Adult domestic rabbit (the same 4 strains) in England
Newborn domestic rabbit
Adult domestic rabbit
Acute course (mild, not contagious)
Adult wild rabbit (natural disease)
Adult domestic rabbit, treated with carcinogens
Neoplastic disease with malignant traits
No disease or mild neoplastic disease
Constitutional
Immunizing
Endocrine
Environmental
Age
Constitutional
Physicochemical
Age

in the adult rabbit by the myxoma variant betoken the same situation for that very similar infection, and it has been shown (22) that no viricidal activity is found in the blood of myxomatous rabbits, and that in rabbits immune to fibroma and infected with myxoma, the antibody reaction against myxoma is much delayed.[2]

If small amounts of virus are injected the rabbit lives long enough to start a defense mechanism, and a progressively growing tumor with characteristics of malignancy may result. It would follow from this that regardless of the amount of virus injected, and of the resistance of the host, the earliest phases of the fibroma infection are always inflammatory, the neoplastic traits not appearing until later and, in fact, this has been observed by several workers (1, 3, 28, 41, 45, 47) and confirmed in our studies.

It would seem logical that in rabbits treated with carcinogens and x-rays a lowering of resistance to the fibroma virus later injected should occur. This was not noticed by Ahlström and Andrews (2), however, probably because they tested their rabbits too late, but it was clearly observed by Clemmensen (11) in his x-rayed rabbits, in which the resistance to reinfection was retarded by several days.

A natural resistance of the rabbit against the virus develops rapidly but gradually after birth. The data in Table II show how delicate is the balance of the two factors, amount of virus and age of the host, upon which the outcome of the disease hangs during the first 15 days of life. Experiment I of Table IV may even suggest an hourly acquisition of resistance. Probably another indication of the delicacy of the two balancing factors is the fact that metastases, the most obvious expression of malignancy, occurred in 7 of 8 cases in rabbits infected from 5 to 24 hours after birth.

The virus in the tumor is protected against the antibody only up to a certain point, because in tumors 25 days old or older the virus was, in general, scanty or even absent (Tables II and III), and in regressing tumors its absence was the rule. In these cases the antibody titers were always very high. In the long run virus in the viscera was incompatible with antibody in the blood, but that there is a phase in the disease where visceral virus and antibody may coexist is plainly shown in the tables.[3]

The factors that influence the effects of the fibroma virus offer many points in common with those that influence avian tumors. Here the causative virus, when injected into newly hatched chicks (13) or embryos (36), brought about a destructive, nonneoplastic condition, the hemorrhagic disease, whereas when injected into older hosts it induced typical sarcomas. These events were determined by factors present in the blood, where, it was shown, a potent virus-neutralizing antibody developed naturally in parallel with the growth of the bird (12); for treatment of chicks with serum from adult chickens brought about a shifting from hemorrhagic into neoplastic lesions, or even a suppression of all lesions (15).[4] The data available on the rabbit fibroma certainly suggest that acquisition by the aging rabbit of an ability to manufacture specific viral antibodies plays a very important part in the defense mechanism. But in the case of the chicken tumors (12) the factors in the blood were present before the virus was injected, whereas in the case of the rabbit fibroma they developed after the virus was inoculated, and one could argue that the viral antibody was merely the result or the by-product of another factor, the one fundamental in defense. The experimental proof that this is not the case is not yet available.[5]

To be sure, other factors may also play a part in the defense; of these, differences in tissue permeability such as are discussed in another publication (16) must be of considerable importance. That differences in the susceptibility of the individual are involved is plainly shown in the tables.

The important conclusion from the present study is that the fibroma virus (a variant of another virus that is highly contagious and pathogenic) can induce neoplasia with traits of malignancy only when the host is endowed with a certain degree of resistance against the virus. If no resistance is present an essentially inflammatory condition develops; if a strong resistance is present a mild disease or no disease at all results.

Analogies and differences between myxoma, fibroma, and malignant growth have been pointed out by

[2] A first conclusion by Shope that antibodies against the myxoma variant are not to be found in the blood of fibroma-immune rabbits (42) was not upheld by other investigators (7, 35), who used a more sensitive technic. However, since Shope's method showed the presence of abundant antibodies against the fibroma virus, the relative difference between the rate of formation of both sorts of antibodies still holds true.

[3] The specific virus-neutralizing antibody is the only one investigated in the present study. Hypothetical factors directed against the fibroma cell as such, independent of the virus infecting it, could perhaps be postulated also as being present in the blood.

[4] The experiments of Blakemore (10) are also of interest in this respect. He obtained a highly virulent strain of chicken lymphomatosis which, when injected into chicks, induced acute necrotizing and inflammatory lesions in the heart and liver, while neoplasia appeared later in the more resistant animals that survived. Lymphomatosis is considered by the author as probably the chronic stage of an acute disease.

[5] It may be useful to recall that the relative resistance of wild rabbits to myxoma virus is not due to viricidal properties in their blood (23, 30). The refractory state is due to those constitutional factors generally included under the term species resistance.

others (2, 37, 42), and the malignant character of the myxoma and the fibroma has been denied by pathologists on morphological grounds (28, 47). If malignant growth is some day shown to be an infectious process the facts here described may contribute to explain its mechanism. In the opposite case they would be useful in that they single out processes that simulate, but are not, malignant neoplasia.

SUMMARY

From the present and a previous investigation on the same subject it appears that following the injection of adult domestic rabbits with the virus of the rabbit fibroma no virus is ever found in the blood, generalized spread of the virus in the viscera occurs only during the first 8 days after infection, immunity to reinfection is already noticeable after 24 hours, and the antibody response is prompt and vigorous.

Following similar injections into newborn rabbits virus is detected in the blood for at least 13 days and in the viscera for at least 21 days after injection, immunity to reinfection is not yet well established 12 days after infection, and the antibody response is slow and poor.

When large amounts of virus are injected into newborn rabbits an acute, lethal disease with traits largely destructive and inflammatory, but also with typical proliferative features, results. This disease shows many analogies with the disease induced in the adult domestic rabbit by another but highly pathogenic virus, that of rabbit myxomatosis, the agent of which is a variant of the fibroma virus.

If small amounts of virus are injected into newborn rabbits a progressively growing tumor results, which induces satellite nodules, sometimes generalized fibromas, and often kills the host. In other cases the tumors regress after having attained a large size. The generalized fibromas are very similar to the generalized lesions obtained by other investigators in rabbits prepared by carcinogens and injected intravenously with fibroma virus.

REFERENCES

1. Ahlström, C. G. The Histology of the Infectious Fibroma in Rabbits. J. Path. & Bact., **46**:461-472. 1938.
2. Ahlström, C. G., and Andrewes, C. H. Fibroma Virus Infection in Tarred Rabbits. J. Path. & Bact., **47**:65-86. 1938.
3. Andrewes, C. H. A Change in Rabbit Fibroma Virus Suggesting Mutation. I. Experiments on Domestic Rabbits. J. Exper. Med., **63**:157-172. 1936.
4. Aragao, de B. H. Sobre o microbio do Myxoma dos coelhos. Brazil Med., **25**:471. 1911.
5. Aragao, de B. H. O virus do mixoma no coelho-domato (*Sylvilagus minenses*), sua transmissão pelos *Aedes scapularis* et *aegypti*. Mem. Inst. Oswaldo Cruz, **38**: 93-99. 1943.
6. Berry, G. P., and Dedrick, Helen M. A Method for Changing the Virus of Rabbit Fibroma (Shope) into that of Infectious Myxomatosis (Sanarelli). J. Bact., **31**: 50-51. 1936.
7. Berry, G. P., and Lichty, J. A., Jr. Immunological and Serological Evidences of a Close Relationship Between the Viruses of Rabbit Fibroma (Shope) and Infectious Myxomatosis (Sanarelli). J. Bact., **31**:49-50. 1936.
8. Berry, G. P. The Transformation of the Virus of Rabbit Fibroma (Shope) into that of Infectious Myxomatosis (Sanarelli). Proc. Amer. Phil. Soc., **77**:473-476. 1937.
9. Berry, G. P. A Non-Lethal "Mutant" Strain of Virus Myxomatosum Derived from Fibroma Virus. J. Bact., **36**:285-286. 1938.
10. Blakemore, F. The Nature of Fowl Paralysis (Neurolymphomatosis). J. Comp. Path. & Therap., **52**:144-159. 1939.
11. Clemmesen, J. The Influence of Roentgen Radiation on Immunity to Shope Fibroma Virus. Am. J. Cancer, **35**:378-385. 1939.
12. Duran-Reynals, F. Neutralization of Tumor Viruses by the Blood of Normal Fowls of Different Ages. Yale J. Biol. & Med., **13**:61-76. 1940.
13. Duran-Reynals, F. A Hemorrhagic Disease Occurring in Chicks Inoculated with the Rous and Fujinami Viruses. Yale J. Biol. & Med., **13**:77-98. 1940.
14. Duran-Reynals, F. Production of Degenerative Inflammatory or Neoplastic Effects in the Newborn Rabbit by the Shope Fibroma Virus. Yale J. Biol. & Med., **13**: 99-110. 1940.
15. Duran-Reynals, F., and Estrada, E. Protection of Chick against Rous Sarcoma Virus by Serum from Adult Chickens. Proc. Soc. Exper. Biol. & Med., **45**:367-372. 1940.
16. Duran-Reynals, F. Tissue Permeability and the Spreading Factor in Infection. A Contribution to the Host : Parasite Problem. Bact. Rev., **6**:197-252. 1942.
17. Findlay, G. M. Notes on Infectious Myxomatosis of Rabbits. Brit. J. Exper. Path., **10**:214-219. 1929.
18. Faulkner, G. H., and Andrewes, C. H. Propagation of a Strain of Rabbit Fibroma Virus in Tissue-Culture. Brit. J. Exper. Path., **16**:271-275. 1935.
19. Gardner, R. E., and Hyde, R. R. Transformation of Rabbit Fibroma Virus (Shope) into Infectious Myxomatosis (Sanarelli). J. Infect. Dis., **71**:47-49. 1942.
20. Haagen, E. Untersuchungen über die übertragbare Myxomatose beim Kaninchen. Mit besonderer Berücksichtigung der Züchtung des Myxomavirus in der Gewebekultur. Zentralbl. f. Bakt. u. Parasitenk., Abt. I. Orig. 121- 1-9. 1931.
21. Haagen, E., und Du Dscheng-Hsing. Weitere Untersuchungen über des Verhalten des Kaninchenmyxomvirus in vitro. Zentralbl. f. Bakt., Abt. I. Orig. **143**:23-31. 1938.
22. Hobbs, J. R. Studies on the Nature of the Infectious Myxoma Virus of Rabbits. Am. J. Hyg., **8**:800-839. 1928.
23. Hobbs, J. R. The Occurrence of Natural and Acquired Immunity to Infectious Myxomatosis of Rabbits. Science, **73**:94-95. 1931.
24. Hoffstadt, R. E., and Pilcher, K. S. Immunological Reaction of Elementary Bodies and of Virus Free Filtrates from Myxoma Infected Chick Membranes with Hyperimmune Rabbit Serum. J. Infect. Dis., **65**:103-112. 1939.
25. Hurst, E. W. Myxoma and the Shope Fibroma. I: The Histology of Myxoma. Brit. J. Exper. Path., **18**:1-15. 1937.

26. Hurst, E. W. Myxoma and the Shope Fibroma. II: The Effect of Intracerebral Passage on the Myxoma Virus. Brit. J. Exper. Path., **18**:15-22. 1937.
27. Hurst, E. W. Myxoma and the Shope Fibroma. III: Miscellaneous Observations Bearing on the Relationship between Myxoma, Neuromyxoma and Fibroma Virus. Brit. J. Exper. Path., **18**:23-30. 1937.
28. Hurst, E. W. Myxoma and the Shope Fibroma. IV: Australian J. Exper. Biol. and Med. Science, **16**:53-64. 1938.
29. Hurst, E. W. Myxoma and the Shope Fibroma. V: Myxoma in the Fibroma-Immune Rabbit, with a Summary of Present Knowledge of the Relationship between Myxoma and Fibroma Viruses. Australian J. of Exper. Biol. and Med. Science, **16**:205-208. 1938.
30. Hyde, R. R., and Gardner, R. E. Infectious Myxomatosis of Rabbits. Am. J. Hyg., **17**:446-465. 1933.
31. Hyde, R. R. Infectious Myxomatosis of Rabbits (Sanarelli) Versus the Fibroma Virus (Shope) with Especial Reference to the Time Interval in the Establishment of Concomitant Immunity. Am. J. Hyg., Sect. B, **30**:47-55. 1939.
32. King, J. W., and Duran-Reynals, F. Development by Ducks of Natural Neutralizing Antibodies for a Duck Variant of the Rous Sarcoma Virus. Yale J. Biol. & Med., **16**:53-57. 1943.
33. Ledingham, J. C. G. Studies on the Serological Inter-Relationships of the Rabbit Viruses Myxomatosis (Sanarelli, 1898), and Fibroma (Shope, 1932). Brit. J. Exper. Path., **18**:436-449. 1937.
34. Lipschuetz, B. Untersuchungen über die Aetiologie der Myxomkrankeit des Kaninchens. Wien. klin. Wochenschr., **40**:1101-1103. 1927.
35. Lush, D. The Serological Relationship of Myxoma and Shope's Fibroma Viruses. Australian J. of Exper. Biol. and Med. Science, **17**:84-88. 939.
36. Milford, J. J., and Duran-Reynals, F. Growth of a Chicken Sarcoma Virus in the Chick Embryo in the Absence of Neoplasia. Cancer Research, **3**:578-584. 1943.
37. Rivers, T. M. Infectious Myxomatosis of Rabbits. Observations on the Pathological Changes Induced by Virus Myxomatosum (Sanarelli). J. Exper. Med., **51**:965-976. 1930.
38. Rivers, T. M., and Ward, S. M. Infectious Myxomatosis of Rabbits. Preparation of Elementary Bodies and Studies of Serologically Active Materials Associated with the Disease. J. Exper. Med., **66**:1-14. 1937.
39. Sanarelli, G. Das myxomatogene Virus. Beitrag zum Studium der Krankheitserreger ausserhalb des Sichtbaren (Vorläufige Mitteilung). Cent. Bakt., Abt. I. Orig. **30**:865-873. 1898.
40. Schlesinger, M., and Andrewes, C. H. The Filtration and Centrifugation of the Viruses of Rabbit Fibroma and Rabbit Papilloma. J. Hyg., **37**:521-526. 1937.
41. Shope, R. E. A Transmissible Tumor-Like Condition in Rabbits. J. Exper. Med., **56**:793-802. 1932.
42. Shope, R. E. A Filtrable Virus Causing a Tumor-Like Condition in Rabbits and Its Relationship to Virus Myxomatosum. J. Exper. Med., **56**:803-822. 1932.
43. Shope, R. E. Infectious Fibroma of Rabbits. III. The Serial Transmission of Virus Myxomatosum in Cottontail Rabbits, and Cross-Immunity Tests with the Fibroma Virus. J. Exper. Med., **63**:33-41. 1936.
44. Shope, R. E. Infectious Fibroma of Rabbits. IV. The Infection with Virus Myomatosum of Rabbits Recovered from Fibroma. J. Exper. Med., **63**:43-57. 1936.
45. Shope, R. E. A Change in Rabbit Fibroma Virus Suggesting Mutation. II. Behavior of the Variant Virus in Cottontail Rabbits. J. Exper. Med., **63**:173-178. 1936.
46. Sprunt, D. H. Infectious Myxomatosis (Sanarelli) in Pregnant Rabbits. J. Exper. Med., **56**:601-608. 1932.
47. Stewart, F. W. The Fundamental Pathology of Infectious Myxomatosis. Am. J. Cancer, **15**:2013-2028. 1931.

Further Observations on the Influence of Simple Caloric Restriction on Mammary Cancer Incidence and Related Phenomena in C3H Mice*

Robert A. Huseby, M.D., Zelda B. Ball, M.S., and
Maurice B. Visscher, Ph.D., M.D.

(From the Divisions of Cancer Biology and General Physiology, Department of Physiology, University of Minnesota, Minneapolis 14, Minnesota)

(Received for publication August 28, 1944)

INTRODUCTION

In a previous report from this laboratory (21) a reduction in the incidence of spontaneous mammary carcinoma in virgin C3H mice from 67 per cent in the control *ad libitum* fed animals to 0 per cent in the experimental animals, which had been maintained from the time of weaning on a diet restricted one-third in calories, was reported. Tannenbaum (19) has described similar effects of caloric restriction. The mechanism by which caloric restriction *per se* produced this striking result was suggested to be endocrine in nature (2). The present report is an attempt to define this mechanism by investigating histologically portions of the endocrine and reproductive systems of those animals still living at the age of 17 to 18 months.

MATERIAL AND METHODS

As the precise method of caloric restriction was given in full in the previous report it suffices here to say that at the time of weaning the restricted animals were placed on a diet that afforded them the same amount of protein, vitamins, and salts as the *ad libitum* fed controls received, but restricted their caloric intake by approximately one-third. On this regime the average body weight of the restricted animals was maintained rather constant throughout life at 12 to 14 gm., while the control animals gained weight almost continuously to average 30 to 32 gm. after 1 year of age.

The animals still living were sacrificed when they reached 17 to 18 months of age; 13 restricted and 11 control animals were thus studied. The ovaries, uteri, mammae, and adrenals were fixed in Bouin's solution, embedded in paraffin, sectioned at 5 microns, and stained routinely with Delafield's hematoxylin and eosin. Whole-mount preparations also were made of some of the mammae from both groups.

* Aided by the Sivertsen Foundation for Cancer Research and the Cancer Research Fund of the Graduate School of the University of Minnesota, and the Works Progress Administration, Project Number 363-65-171-140.

RESULTS

At autopsy 1 of the control animals was found to have a mammary carcinoma, thus raising the final tumor incidence of the control group to 72 per cent, while that of the restricted group remained at 0 per cent.

Ovaries.—The ovaries of the control animals, as would be expected with advanced age, are composed mostly of stroma with but very few follicles (Fig. 1). The follicles present are maturing follicles, containing much follicular liquor and a normal appearing granulosa. Younger follicles are rather scarce, and primary follicles very rare. Instances of ovum degeneration with luteinization of the granulosa can be noted, as well as an occasional rather typical corpus luteum. The stroma of these ovaries is composed largely of interstitial cells, large yellow cells containing lipoid, and connective tissue "stromal cells" supporting the other elements being especially numerous about the blood vessels. The interstitial cells (Fig. 2) are eosin-staining cells, morphologically similar to lutein cells and, in the mouse, apparently may develop from lutein cells of either the corpora lutea or luteinized follicles as well as from the stromal connective tissue cells. The precise nature and origin of the large "lipoid cells" we do not know (Figs. 1, 2, 3, and 4). They appear to be multilocular fat cells, with the nucleus usually pushed to one side and the cytoplasm containing many fat droplets. The cytoplasm between the droplets appears yellow in hematoxylin-eosin preparations and stains dark with iron hematoxylin. These cells apparently develop and increase in number with advancing age, for although they form large clusters in all the ovaries of both the control and restricted animals of this experiment, in the stock colony of C3H mice they are to be found only singly, or not at all, in ovaries of mice up to 260 days of age, but are seen to form small clusters in animals 300 to 350 days old. Similar cells are occasionally noted also in the

uterine endometrium of the mice 17 to 18 months of age.

In sharp contrast to the picture described above, the ovaries of the restricted animals are smaller in size, filled with follicles, and possess no interstitial cells (Fig. 3). Although weights were not taken, these ovaries appeared to be about one-third smaller than those of the control group. Follicles occupy much of these ovaries, and the younger follicles are in preponderance. Primary follicles are numerous, especially just under the germinal epithelium, and many developing follicles, containing much granulosa but relatively little liquor, can be seen throughout the cortices. Follicular degeneration is common, and consists of two types: (a) Most prevalent in the smaller follicles in which the ovum has degenerated is the transformation of the granulosa cells to "wheel cells" (cells with heavy chromatin in a "spoke-like" arrangement); the nests of wheel cells thus formed become broken up by connective tissue strands and enter into formation of the general stroma of the gland (Fig. 4); (b) degeneration of larger follicles occurs through their invasion by a mass of large leptochromatic connective tissue cells, with gradual disappearance of the granulosa cells (Fig. 3). This process of connective tissue invasion can occur either before or after the ovum has degenerated; thus many medium-sized follicles present in the ovaries are composed of theca and normal granulosa but contain no ova. The stroma of the ovaries is very compact and composed of wheel cells, stroma connective tissue cells, and the large lipoid cells noted in the control group. The germinal epithelium is cuboidal to low columnar, with a goodly number of mitotic figures and evidence of the production of many ova.

There was one noteworthy exception to the above general descriptions in the control animal that had a mammary carcinoma at the time of sacrifice. The morphology of its ovaries appears to be a combination of the two pictures described above. There are but few follicles, as is characteristic of the control animals, but the stroma is compact and contains many wheel cells. Interstitial cells are lacking throughout the stroma, although a few small corpora lutea are present. There are several corpora albicantia in this ovary; these are not common in the general control group and are entirely absent in the restricted animals. Thus it would appear that in this animal, because of the cachexia accompanying the malignant growth, the normal ovarian picture has been varied toward that of caloric restriction. It is further to be noted that the uterus of this animal is the smallest and least developed of any in the control group (Table I).

Uteri.—The uteri of the two groups of animals are as different as are the ovaries. The first striking difference being in the comparative sizes, the middle one-third of the uterine horns was used for sectioning to obtain comparable areas for measurement. The greatest cross sectional diameter and the diameter at right angles to it were measured by means of an ocular micrometer, and the approximate areas calculated ($A = \pi ab$, where a and b are hemidiameters). The cross sectional areas of the control uteri average 11.1 sq. mm. (Table 1), as contrasted to 0.504 sq. mm. for the restricted (Table II). In other words, the cross sectional area of the uteri of the control animals is roughly 20 times that of the restricted animals as compared to general body weights of approximately $2\frac{1}{2}$ to 1.

TABLE I: DIMENSIONS OF UTERI OF CONTROL MICE

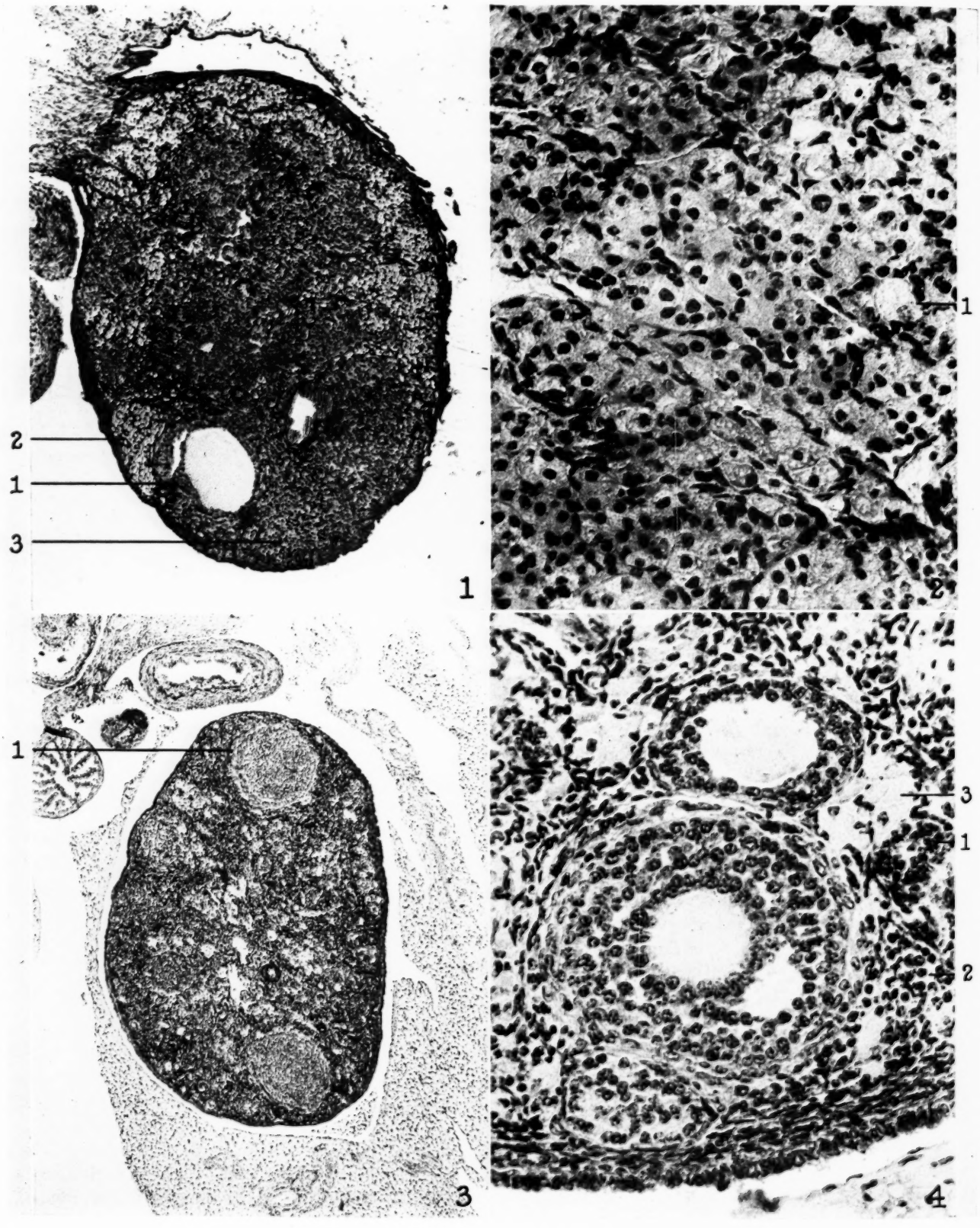
Hemiaxes measured by an ocular micrometer, and areas calculated using the formula $A = \pi ab$, where a and b are the hemiaxes.*

Animal no.	Hemiaxes in mm.		Area in mm.²	Average area
	Major	Minor		
4	2.15	1.73	11.7	10.0
	1.83	1.44	8.27	
10	2.11	1.89	12.6	11.0
	2.12	1.42	9.45	
62	2.80	2.15	18.9	19.0
	2.63	2.31	19.1	
64	2.05	1.46	9.4	10.1
	1.98	1.75	10.9	
72	1.69	1.51	8.02	8.0
74	2.17	1.53	10.43	10.4
92	2.40	1.88	14.2 (Photo)	11.4
	1.73	1.56	8.5	
96	2.01	1.66	10.5	10.5
98	2.76	2.09	18.1	18.1
100	1.77	1.25	6.95	6.95
102	1.50	1.26	5.95	6.3 (Tumor)
	1.58	1.33	6.6	
Total average				11.1 mm.²

* Where two values are given for the same mouse, both right and left uterine horns were measured.

The histological differences are also distinct. The endometria of the control animals contain a great number of glands, many of which are dilated (Fig. 5). The epithelium of the glands is generally low columnar, with a tendency in many areas toward "multilayering" of the nuclei. Mitoses occur commonly (Fig. 6). The stroma is generally loose, and mitotic figures are scattered throughout. This endometrial picture would be consistent with that produced by prolonged estrogenic stimulation. The muscle cells of the myometrium have large blunt nuclei surrounded by rather abundant cytoplasm, giving the myometrium a somewhat loose appearance.

The restricted endometria are, on the other hand, very undeveloped (Fig. 5). The glands are relatively



FIGS. 1-4

few in number, very small, and without secretion, and the epithelium lacks evidence of proliferation (Fig. 7). The stroma is very compact and without mitoses. Just under the surface epithelium, cells with a nuclear pattern very similar to, if not identical with, the wheel cells of the ovary are prevalent, sometimes forming rather large clusters. The myometrium is very compact, the muscle nuclei small, closely packed, and irregular with a tendency toward pointed ends. The general appearance of these uteri is much the same as that in castrate animals.

TABLE II: DIMENSIONS OF UTERI OF RESTRICTED MICE

Hemiaxes measured by an ocular micrometer, and areas calculated using the formula $A = \pi ab$, where a and b are the hemiaxes.*

Animal no.	Hemiaxes in mm. Major	Minor	Area in mm.²	Average area
3	.382	.351	.422	.410
	.412	.307	.398	
9	.487	.351	.537	.465
	.395	.316	.392	
11	.438	.425	.585 (Photo)	.566
	.495	.351	.546	
31	.371	.320	.373	.373
35	.456	.359	.515	.515
37	.835	.421	1.104	1.104
39	.470	.250	.370	.383
	.417	.302	.396	
45	.351	.241	.266	.308
	.351	.316	.349	
49	.416	.311	.406	.397
	.470	.263	.388	
55	.460	.307	.444	.530
	.487	.403	.616	
79	.342	.254	.273	.273
81	.636	.483	.965	.907
	.565	.478	.849	
83	.346	.294	.320	.320
Total average				.504 mm.²

* Where two values are given for the same mouse, both right and left uterine horns were measured.

Mammary gland.—The whole-mount preparations of the mammary glands showed the mammae of the restricted animals to be considerably less developed than those of their *ad libitum* fed controls (Figs. 8 and 9). Not only was the over-all size of the glands definitely less, but the general glandular architecture was much simpler than in the controls. There were fewer large ducts per unit area, smaller ducts were greatly decreased in number, and the contour of the ducts in general was smoother, there being few lateral alveolar buds. This general underdevelopment plus the extreme paucity of lateral alveolar buds suggest a much lower level of hormonal stimulation in the restricted animals. In spite of this underdevelopment, however, areas of hyperplastic alveolar development were found in the restricted as well as in the control animals.

Adrenals.—As castration of C3H mice leads to adrenal cortical hyperplasia with the production of extragonadal female sex hormones, the adrenal glands were studied, but no instance of hyperplasia was found in either group. The differences in the adrenal cortices are not great. The cortical cells in the restricted appear to contain somewhat less lipoid, as judged by the decreased vacuolization of their cytoplasm, and the zona glomerulosa appears less well differentiated than in the control animals, but no very outstanding difference is to be noted. We found no wheel cells in the restricted adrenals.

DISCUSSION

The histological picture found in the genitalia of old virgin mice maintained from the time of weaning on a caloric intake two-thirds that of the control *ad libitum* fed animals is essentially that of hypophysectomy, as described by Smith (17) and by Selye, Collip, and Thomson (18); and of "pseudohypophysectomy" due to chronic inanition, described by Moore and Samuels (11) and by Mulinos and Pomerantz (12, 14). The ovaries contain no corpora lutea but have numerous so-called wheel cells throughout the stroma, and the uteri have essentially the appearance found in castrate animals but also exhibit wheel cells. The only point in which the picture found here differs from that generally described for either hypophysectomy or pseudohypophysectomy is in the relatively

DESCRIPTION OF FIGURES 1 TO 4

FIG. 1.—Typical section through ovary of control mouse. Only one large follicle present [1], most of gland being composed of interstitial cells [3] and large "lipoid cells" [2] supported by connective tissue stroma. Mag. × 30.

FIG. 2.—Stroma of ovary in Fig. 1, under higher magnification. Morphology of "lutein-like" interstitial cells can be seen, as well as that of a few of the large "lipoid cells" [1]. Mag. × 400.

FIG. 3.—Typical section through ovary of calorie-restricted mouse. Note particularly the large number of follicles visible, the 2 large follicles exhibiting an overproduction of granulosa with invasion of one of these follicles by connective tissue [1], and compactness of stroma in general. Mag. × 30.

FIG. 4.—Stroma of ovary in Fig. 3, with higher magnification. Two normal follicles seen, as well as a degenerating follicle with granulosa transforming into "wheel cells" [1] and a nest of "wheel cells" being broken up by connective tissue [2]. Large "lipoid cells" [3] contrast sharply with small stromal connective tissue cells and "wheel cells." Mag. × 400.

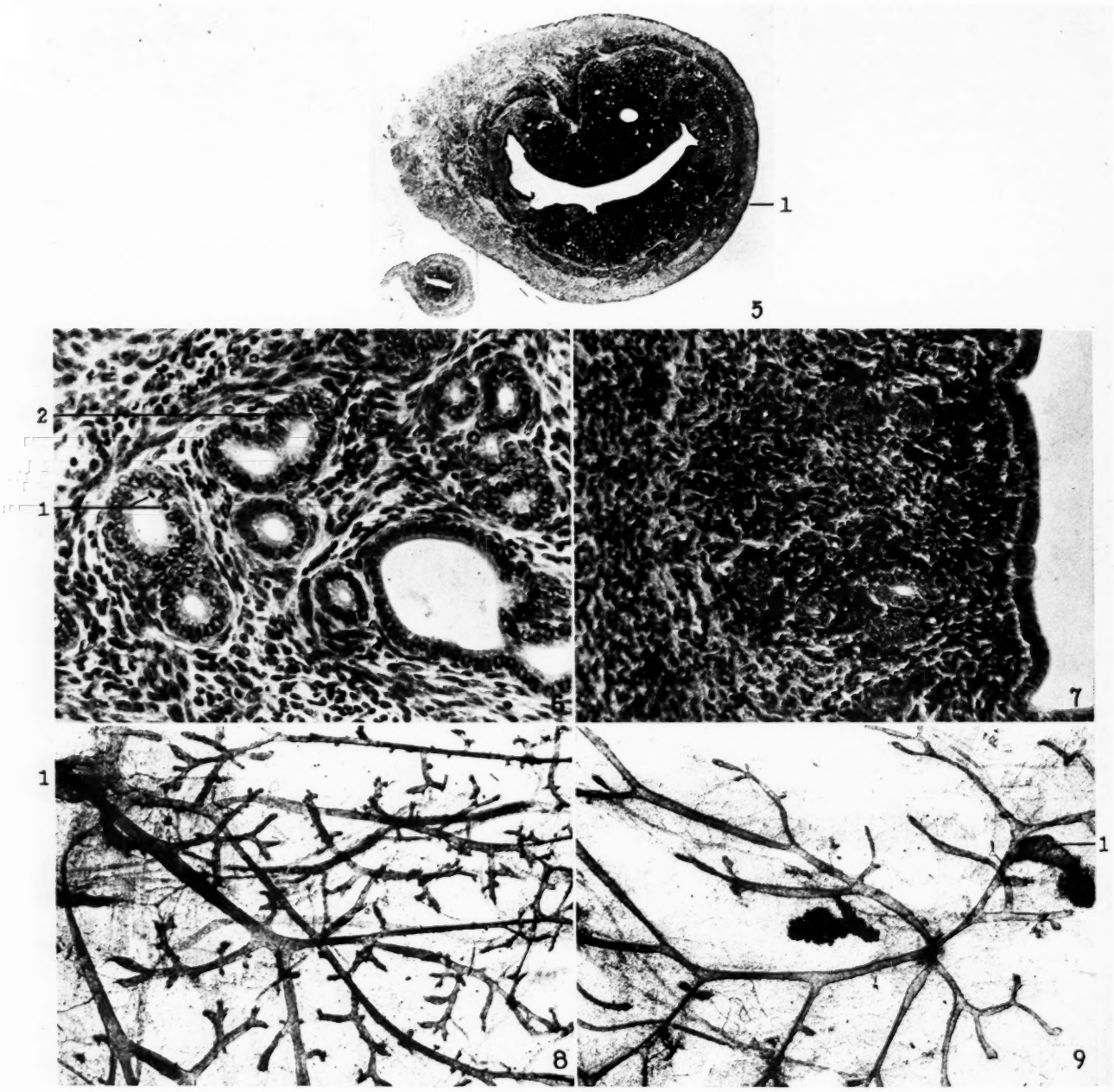


FIG. 5.—Typical uteri of control and restricted animal under identical magnification. For actual measurements see Tables I and II. Note particularly the large amount of endometrium containing some large dilated glands in control uterus [1]. Mag. × 12.4.

FIG. 6.—Endometrium of control uterus of Fig. 5; higher magnification. Area does not include any dilated ducts, but looseness of stroma, mitoses of glandular epithelium [1], and tendency toward "multilayering" of epithelial nuclei [2] can be seen. Mag. × 330.

FIG. 7.—Endometrium of restricted uterus of Fig. 5; higher magnification. Note especially thinness of endometrium, small size of glands, and compactness of stroma. Mag. × 330.

FIG. 8.—Typical area of mammary gland whole mount from control animal. Field includes primary duct [1], and shows a well developed mammary gland with many small side ducts and lateral alveolar buds. Mag. × 10.3.

FIG. 9.—Typical area of mammary gland whole mount from restricted animal. Although field includes same actual area as Fig. 8, because of the much smaller size of the restricted glands a greater percentage area is shown. Note primary duct [1] and paucity of small side ducts and lateral alveolar buds. A rather large "precancerous" area of alveolar hyperplasia in center of field. Mag. × 10.3.

greater number of follicles present in the ovaries of the restricted group as compared to those of the control animals. It is to be remembered, however, that the conditions of this experiment differed in two essentials from those previously described in the literature. The animals in this study were placed on the restricted regime before puberty instead of later, and the organs were not taken for study until the animals were 17 to 18 months of age, when the control animals were well past their "fertile" period and their ovaries would be expected to be almost devoid of follicles.

Hypophyseal insufficiency apparently affects mammary growth in two ways. First, there is a decrease in the production of ovarian hormones, as indicated by a cessation of estrus and an adnexal atrophy (10, 12, 14). In underfeeding, as in hypophysectomy, it has been reported that both these changes may be corrected by supplying the animal with impure gonadotropic hormone (10, 13, 17). Second, in underfeeding the response of the mammary gland to a given amount of estrogen is considerably decreased (1, 20), while total hypophysectomy renders the mammae almost completely refractory to large doses of estrogenic substances (6, 9, 16), unless an extract from the anterior pituitary is supplied (7, 15). Thus, Trentin and Turner (20) have shown that as the level of food intake is decreased, proportionately greater amounts of estrogenic substance are necessary to produce duct growth in male mice. With a one-third reduction of diet, using 3 gm. daily as normal, they found it necessary to increase the total estradiol benzoate injection from 0.083γ to 0.415γ in order to obtain an assay response. In accord with the observations cited above, the mammae of the restricted animals of this study are notably underdeveloped. They are much smaller in over-all size than those of the control group, contain fewer ducts per unit area, especially fewer ducts of smaller caliber, and the contour of their ducts in general is smooth, there being few lateral alveolar buds. All these features reflect a lowered mammary response.

It thus appears that the reduction in mammary carcinoma noted in this experiment can be adequately explained on a hormonal basis. As castration of young mice (for literature see 8) in the absence of adrenal hyperplasia (24) causes a lowering in the incidence of mammary cancer due to a decrease in ovarian hormones, so caloric restriction *per se* reduces the occurrence of mammary carcinoma in mice by producing hypophyseal insufficiency, which decreases not only the production of ovarian hormones but also the response of the mammae to what estrogen is present in the circulation.

The work of White and Andervont (22) and White and White (23) is in agreement with this concept. By feeding a diet low in cystine to virgin mice of Andervont's subline of C3H, they were able to reduce the incidence of mammary carcinoma from 97.6 per cent in the control animals to 0 per cent in the experimental group. It is of interest to note that similar to animals on a calorie-restricted diet, the animals on the low cystine diet weighed on the average 12.4 gm. at 6 months of age as compared to 25.3 gm. for the control group, and also exhibited estrus only infrequently. When pellets of diethylstilbestrol and cholesterol were placed subcutaneously so that the animals on both the low and high cystine diet were maintained in continuous estrus, the incidence of mammary carcinoma was 44.7 per cent for the animals on the low cystine as compared to 92 per cent for the control animals. Thus is would appear that by increasing estrogenic stimulation to levels above normal it was possible to overcome only partially the effects of the low cystine diet, as would be expected if this diet caused a pituitary insufficiency as suggested by the anestrus and other changes.

The occurrence of "precancerous" areas of alveolar hyperplasia in the mammae of the restricted animals is somewhat difficult to explain, in view of the present knowledge of this lesion. Gardner (4) has observed them to be more frequent in strains with a high incidence of mammary tumors, and more frequent in older mice. However, in virgin mice with the milk influence and genetic constitution for spontaneous mammary tumor development, but with a low incidence of mammary carcinoma because of a low hormonal stimulation, *e.g.,* strain A mice, few if any of these nodules occur (3). Gardner (5) found that hypophysectomy of old tumorous mice did not cause these hyperplastic nodules to atrophy or prevent some from developing into carcinomas. No work has been done on the role of the pituitary in producing these "precancerous" lesions, but why they should develop in the presence of such a low level of hormonal stimulation and, once developed, why they did not become cancerous at least in some of the restricted mice, is difficult to understand.

Although the calorie-restricted animals in this study received amounts of protein identical to that consumed by their controls, the fact must be remembered that in the absence of adequate calories from other sources amino acids are utilized for fuel, and that therefore a simple caloric restriction can indirectly produce amino acid deficiencies. Whether part of or all the effects produced by caloric restriction in these experiments were due to such an indirect effect of increasing amino acid requirements cannot be decided on the basis of any data now at hand.

SUMMARY

1. The final incidence of mammary carcinoma in virgin C3H mice maintained from weaning on a diet supplying the same amounts of protein, vitamins, and salts as an *ad libitum* fed control group received, but restricting the caloric intake by one-third, was 0 per cent as compared to 72 per cent for the control animals.

2. The histological picture of the ovaries, uteri, and mammae of the restricted animals was that of pseudo-hypophysectomy due to inanition.

3. The mechanism by which caloric restriction *per se* reduces the incidence of mammary carcinoma is probably pituitary insufficiency producing: (a) a lowered level of ovarian secretion; (b) a relative refractoriness of the mammary gland to estrogenic substances.

REFERENCES

1. Astwood, E. B., Geschickter, C. F., and Rausch, E. O. Development of the Mammary Gland of the Rat. Study of Normal, Experimental and Pathologic Changes and Their Endocrine Relationships. Am. J. Anat., **61**: 373-405. 1937.
2. Ball, Zelda B., Huseby, R. A., and Visscher, M. B. The Influence of Caloric Restriction on Ovarian Function in Relation to Mouse Mammary Cancer Incidence. American Association for Cancer Research, Inc., 35th Annual Meeting. 1942. Abst. in Cancer Research, **3**:131. 1943.
3. Bittner, J. J., Huseby, R. A., Visscher, M. B., Ball, Zelda, B., and Smith, F. Mammary Cancer and Mammary Structure in Inbred Stocks of Mice and Their Hybrids. Science, **99**:83-85. 1944.
4. Gardner, W. U., Strong, L. C., and Smith, G. M. The Mammary Glands of Mature Female Mice of Strains Varying in Susceptibility to Spontaneous Tumor Development. Am. J. Cancer, **37**:510-517. 1939.
5. Gardner, W. U. Persistence and Growth of Spontaneous Mammary Tumors and Hyperplastic Nodules in Hypophysectomized Mice. Cancer Research, **2**:476-488. 1942.
6. Leonard, S. L., and Reece, R. P. Failure of Steroid Hormones to Induce Mammary Growth in Hypophysectomized Rats. Endocrinology, **30**:32-36. 1942.
7. Lewis, A. A., Gomez, E. T., and Turner, C. W. Mammary Gland Development with Mammogen I in the Castrate and the Hypophysectomized Rat. Endocrinology, **30**: 37-47. 1942.
8. Loeb, L. The Significance of Hormones in the Origin of Cancer. J. Nat. Cancer Inst., **1**:169-195. 1940.
9. Lyons, W. R., and Pencharz, R. I. Reactions of Mammary Glands of Normal and Hypophysectomized Male Guinea Pigs to Female Sex Hormone. Proc. Soc. Exper. Biol. & Med., **33**:589-592. 1936.
10. Marrian, G. F., and Parkes, A. S. The Effect of Anterior Pituitary Preparations Administered during Dietary Anoestrus. Proc. Roy. Soc., s. B, **105**:248-258, 1929.
11. Moore, C. R., and Samuels, L. T. The Action of Testes Hormone in Correcting Changes Induced in the Rat Prostate and Seminal Vesicles by Vitamin B Deficiency or Partial Inanition. Am. J. Physiol., **96**:278-288. 1931.
12. Mulinos, M. G., and Pomerantz, L. Pseudo-Hypophysectomy: A Condition Resembling Hypophysectomy Produced by Malnutrition. J. Nutrition, **19**:493-504. 1940.
13. Mulinos, M. G., and Pomerantz, L. The Reproductive Organs in Malnutrition: Effects of Chorionic Gonadotropin upon the Atrophic Genitalia of Underfed Male Rats. Endocrinology, **29**:267-275. 1941.
14. Pomerantz, L., and Mulinos, M. G. Pseudo-Hypophysectomy Produced by Inanition. Am. J. Physiol., **126**: 601. 1939.
15. Reece, R. P., and Leonard, S. L. Effect of Estrogens, Gonadotropins and Growth Hormone on Mammary Glands of Hypophysectomized Rats. Endocrinology, **29**: 297-305. 1941.
16. Samuels, L. T., Reinecke, R. M., and Peterson, W. E. Relation of Nutrition to Mammary Growth after Estradiol Administration to Hypophysectomized Rats. Proc. Soc. Exper. Biol. & Med., **46**:379-382. 1941.
17. Smith, P. E. Hypophysectomy and a Replacement Therapy in the Rat. Am. J. Anat., **45**:205-273. 1930.
18. Selye, H., Collip, J. B., and Thompson, D. L. On the Effect of the Anterior Pituitary-Like Hormone on the Ovary of the Hypophysectomized Rat. Endocrinology, **17**:494-500. 1933.
19. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction *per se*. Cancer Research, **2**:460-467. 1942.
20. Trentin, J. J., and Turner, C. W. Quantitative Study of the Effect of Inanition on Responsiveness of the Mammary Gland to Estrogen. Endocrinology, **29**:984-989. 1941.
21. Visscher, M. B., Ball, Zelda B., Barnes, R. H., and Silvertsen, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.
22. White, J., and Andervont, H. B. Effect of a Diet Relatively Low in Cystine on the Production of Spontaneous Mammary-Gland Tumors in Strain C3H Female Mice. J. Nat. Cancer Inst., **3**:449-451. 1943.
23. White, F. R., and White, J. Effect of Diethylstilbestrol on Mammary Tumor Formation in Strain C3H Mice Fed a Low Cystine Diet. J. Nat. Cancer Inst., **4**:413-415. 1944.
24. Woolley, G., Fekete, E., and Little, C. C. Effect of Castration in the Dilute Brown Strain of Mice. Endocrinology, **28**:341-343. 1941.

Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in Spayed C3H Mice

Edward W. Wallace, M.D.,* Helene Wallace,** and C. A. Mills***

(From the Laboratories for Pharmacology and Experimental Medicine, University of Cincinnati, Cincinnati 29, Ohio)

(Received for publication August 14, 1944)

In a recent report (1) we presented evidence of an increased incidence, earlier appearance, and a more rapid growth of spontaneous mammary tumors in virgin C3H mice of Andervont's subline strain subjected to stimulating coolness (68° F. as contrasted with 91° F.). That study has now been extended to cover spayed females of the same strain, with results that confirm those obtained on the unspayed virgin females.

The mice were raised in an air conditioned room at 79° F., the females spayed at 20 to 25 days of age, and those of each litter divided between the three rooms (68° F., 79° F., and 91° F.) when about 2 months of age. Thus for every spayed female in one room there was another of the same litter in each of the other rooms. Purina dog chow checkers were used as the sole food throughout the experiment.

The accompanying table sets forth the summarized findings. Those mice dying before the earliest tumor age was reached were not included in the calculations, nor were those dying of accident at any time (accidental death most often being due to getting a leg caught in the wire-mesh floor of the cage). Most of the uncounted deaths were of this accidental type and were more frequent among mice of the 91° F. room, perhaps because of their smaller size.

Tumors appeared earliest in mice of the 68° F. room, next at 79° F., and at the latest mean age in the 91° F. room. The difference in age at tumor appearance between mice of the 68° F. and 91° F. rooms (2.32 ± 0.94 months) is over twice as great as was found for analogous groups of the unspayed mice, but it fails to reach definitely significant proportions because of the small numbers of mice involved. The difference is 2.5 times its own probable error, and would occur by chance alone once in 10 times. The percentage of mice developing tumor showed a similar ranking of the three groups, with 22.5 per cent at 68° F., 21.7 per cent at 79° F., and 17.1 per cent at 91° F. In our previous report on unspayed mice, the tumor incidence at 68° F. was 44 per cent higher than at 91° F., while the spayed mice at 68° F. here show a 32 per cent greater prevalence. In the present series the only instances of multiple tumors occurred in the 68° F. group.

SPONTANEOUS TUMORS IN SPAYED C3H MICE

	At 68° F.	At 91° F.	At 79° F.
Number of mice spayed	60	60	60
Number included in calculations	49	41	46
Number developing tumors	11 (2 multiple)	7 (no multiple)	10 (no multiple)
Mean age (months) at tumor appearance	15.32 ± 0.80	17.64 ± 0.50	16.90 ± 0.71
Mean age (months) at death of nontumorous	19.74 ± 0.44	18.53 ± 0.38	20.22 ± 0.41
Percentage tumors developing	22.5	17.1	21.7

Although spayed mice show almost the same percentage accentuation of neoplasia as the unspayed in a cool environment (68° F. as compared to 91° F.), all groups of the spayed exhibit a lower incidence and later mean age for tumor appearance than did analogous groups of the unspayed. It would thus seem that removal of the ovarian influence lessens the tendency to neoplasia, but probably does not interfere with the effect of environmental temperature.

CONCLUSIONS

Spayed C3H mice of the Andervont subline strain exhibit a lowered tendency to neoplasia, but they show almost the same stimulating influence (on a

* Associate Professor of Pharmacology, University of Cincinnati (deceased).

** Research Associate, University of Cincinnati.

*** Professor of Experimental Medicine, University of Cincinnati.

percentage basis) of a cool environment that was previously noted in the unspayed. Thus the higher fertility and greater ovarian activity exhibited by unspayed animals adapted to cold (2) certainly do not seem wholly responsible for the increased tendency to neoplasia under such environmental conditions.

REFERENCES

1. Wallace, E. W., Wallace, Helene, and Mills, C. A. Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in C3H Mice. Cancer Research, **4**:279-281. 1944.
2. Ogle, Cordelia. Adaptation of Sexual Activity to Environmental Stimulation. Am. J. Physiol., **107**:628-634. 1934.

Estimation of Urinary 17-Ketosteroids in the Diagnosis of Adrenal Cortical Tumors*

F. L. Warren, Ph.D.

[*From The Chester Beatty Research Institute, The Royal Cancer Hospital (Free), London, England*]

(Received for publication July 20, 1944)

In 1932 Cushing (6) described a syndrome, now known by his name, which he ascribed to the presence of an adenoma of the basophil cells of the anterior part of the pituitary gland. This syndrome is characterized by: (a) a rapidly acquired adiposity, confined to face, neck, and trunk; (b) a tendency to become round-shouldered, even to measurable loss of height; (c) a sexual dystrophy, shown by early amenorrhea in women and ultimate functional impotence in men; (d) a tendency to hypertrichosis of face and trunk in females and preadolescent males; (e) a dusky or plethoric appearance of the skin, with purplish *linae atrophicae*; (f) various other symptoms, including hypertension, abdominal pains, fatiguability, and ultimately extreme weakness. Frequently there is no definite lesion of the other endocrine glands, although the adrenals are generally enlarged and may contain small adenomas, regarded by Cushing as secondary. The syndrome appears to be more common in women than in men.

A syndrome closely resembling that described by Cushing arises in patients suffering from adrenal cortical tumor. The clinical manifestations depend on the sex and age of the patient. In young and adolescent boys the "herculean" type results. There is precocious growth, and early muscular development, ossification, and dentition. Hair appears early on the pubis, face, and body. The external genitalia enlarge to adult size. The condition is very rare in adult males and, in these cases, the development tends towards the female type. The breasts enlarge and even secrete milk; the testes atrophy; there is loss of libido and development of the female type of obesity. If the adrenal tumor occurs in a young girl before puberty, changes towards the adult type are noticed. There is fatness; hair appears on the pubis and sometimes on the face; the skin is red and coarse; the vocal cords enlarge and the voice coarsens; ossification and dentition may be hastened; the clitoris may become much enlarged, and menstruation does not occur at puberty. The presence of a tumor in a female after puberty leads to changes of a similar nature. The first symptom is frequently the cessation of menstruation. Hirsutism occurs and the pubic hair assumes a male distribution. *Striae atrophicae* appear on the abdomen, hips, and thighs. The clitoris and labia enlarge; the uterus and ovaries may atrophy; the breasts diminish in size. There is considerable obesity with fat distributed on abdomen, chest, buttocks, and hips. Hypertension may be present. Another type exists, which shows amenorrhoea, obesity, hypertrichosis, diabetes, and hypertension.

Probably no true differentiation can be made between the syndrome associated with a basophil adenoma of the pituitary gland and that associated with an adenoma (or malignant tumor) of the adrenal cortex. The syndromes of the two are practically identical, especially in women. In each type, whether a basophil adenoma is present or not, Crooke (4) has shown that the basophil cells exhibit a characteristic hyaline change, which he regards as the common pathological denominator in all forms of the Cushing syndrome or "basophilism."

The differential diagnosis between basophilism associated with an adrenal cortical tumor and other forms of basophilism on clinical grounds alone is difficult. Occasionally the presence of an adrenal tumor can be determined by palpation, or it may be recognized by x-ray photography after perirenal insufflation of air. Kepler, Walters, and Piper (9) suggested that the diagnosis of adrenal cortical tumor in females could be based on the recognition of enlargement of the clitoris, masculinization, and the fact that in these cases the obseity is not of the "buffalo type" with a cervical hump of fat. Cases occur, however, in which these clinical signs are absent.

In view of these diagnostic difficulties the evidence brought forward by Crooke and Callow (5) that differentiation of the adrenal cortical tumor group may be made by estimating the greatly increased excretion of urinary androgens in these cases is clearly of great importance. In general all later work has confirmed this suggestion.

In using this method 3 problems arise. (a) What is the lower limit of ketosteroid excretion that may be taken as indicating the presence of an adrenal cortical

* Because of the difficulties of international communication the author has not read proof of this article.

tumor? (b) Do other tumors or diseases lead to increased excretion? (c) Does a low ketosteroid excretion necessarily exclude the presence of an adrenal neoplasm? Partial answers to these questions are already possible, but more definite statements must clearly wait upon the publication of more numerous data. The results recorded in the present paper are a contribution to this.

The cases described have been investigated during the past 6 years. In 1938 Dr. R. K. Callow very kindly demonstrated his methods to me before the publication of his paper (2), and the same method has been used since as a routine test for the presence of adrenal cortical tumors.

METHODS AND MATERIALS

A complete 24 hour collection of urine was made whenever possible. Although the daily excretion of 17-ketosteroids in any individual is subject to variation, experience gained in repeated determinations on both normal and diseased subjects showed that an isolated determination is almost invariably sufficient to indicate the *level* of excretion for diagnostic purposes.

An aliquot of the urine (100 ml.) was acidified with concentrated hydrochloric acid (15 ml., sp. gr. 1.16), benzene was added (50 ml.), and the mixture refluxed on a water bath for 4 to 6 hours. The mixture was then cooled, and the benzene layer separated off and replaced by fresh benzene (50 ml.). The mixture was again refluxed for 4 to 6 hours. Any further extractions removed negligible amounts of ketosteroids. The combined benzene extracts were washed successively with water (100 ml.), 2*N* NaOH (4 times with 50 ml. portions), *N* HCl (100 ml.), and water (100 ml.). The washed benzene solution was filtered through paper and the benzene distilled off on a steam bath. The last traces of solvent were removed by evaporation under reduced pressure. To the dry residue absolute ethyl alcohol (4 ml.) was added, and solution insured by gentle warming. The alcoholic solution was filtered through paper and was then ready for colorimetry. Two-tenths milliliter of alcoholic solution (equivalent to 5 ml. of the original urine) was used for each determination. All estimations were carried out in duplicate. The colorimetric estimation of the 17-ketosteroids in the extract was made by the method of Callow, Callow, and Emmens (2). From time to time various modifications of this method that have appeared in the literature were tried, but nothing superior to it for routine use was found.

A large number of the cases investigated were females in which the possibility of an adrenal tumor could not be excluded on clinical grounds alone. Cases of other types and from both sexes were included in the series in order to widen the search for exceptional or anomalous excretions that would be of importance in assessing the value of the method as a specific diagnostic test for adrenal cortical tumor.

RESULTS

The values for the urinary excretion of 17-ketosteroids in the cases studied are shown in the tables.

In Table I the division of the cases into "pituitary basophilism," "adrenal virilism," and "hirsutism" is admittedly arbitrary. The nature of the material was such that no accurate diagnosis had been made at the time of the estimation of 17-ketosteroids. As a rough guide, those cases in which the balance of the clinical findings suggested that the primary disorder was pituitary in origin, were classified as "pituitary basophilism," while cases in which it was suggested that the adrenal cortex was primarily responsible for the symptoms were grouped under "adrenal virilism." Cases briefly designated as "hirsutism" were those in which the main feature was hypertrichosis, not necessarily of male distribution, and in which other signs of masculinization were not predominant. The excretions of 17-ketosteroids found in 4 proved cases of carcinoma of the adrenal cortex in women are shown in the last 4 entries of Table V.

DISCUSSION

(a) *Adrenal tumor cases.*—The values found in 4 cases of carcinoma of the adrenal cortex (Table V, last 4 entries) were extremely high, the lowest being 83 mgm. and the highest 690 mgm. per day. Details of the individual cases were as follows:

Case 66.—(B. A.) Married woman aged 54. Admitted to hospital after 3 weeks' duration of melancholia and manic depression. Her complexion was florid and there was advanced hirsutes, with a heavy moustache and beard and a male distribution of pubic hair. The patient stated that she had had abdominal swelling for 2 years. On laparotomy a left upper abdominal tumor was disclosed, which extended into the loin and down to the iliac crest. A biopsy specimen showed sheets of clear cells with uniform rounded nuclei, and the diagnosis of cortical carcinoma of the adrenal gland was made. The patient developed cough, dyspnea, and cyanosis 2 weeks after the operation and died 9 days later. Postmortem examination confirmed the diagnosis.

Case 75.—(E. V.) Woman aged 42. Complained of hirsutism and increasing weight. Her skin was dry and her hair falling out. The patient suffered from headaches and palpitations. Climacteric had occurred 1 year previously and was followed by edema of feet and legs. On laparotomy a large mass was palpated above the right kidney. In a subsequent operation the

right kidney was exposed and a large vesicular mass lying above the kidney was removed. Sections [1] of this mass showed it to be a carcinoma of the adrenal cortex. The patient died soon after operation.

Case 79.—(A. M.) Woman aged 49. Diagnosed clinically as probable adrenal tumor and operation decided upon. Eight days before removal of the tumor the excretion of 17-ketosteroids was found to be 269 mgm. per day. At operation a large tumor [1] of the adrenal cortex was removed. The excretion fell to 187 mgm. per day in the 24 hours after operation. The patient made an excellent recovery from the operation and 10 weeks later her excretion had fallen to a normal value of 7.7 mgm. per day.

fraction by condensation with succinic anhydride and subsequent purification of the half-succinates. From both urines (Cases 75 and 79) the main product was the half-succinate of dehydro*iso*androsterone. Attempts to isolate androsterone from this fraction were unsuccessful.

References to 20 other cases of adrenal cortical tumor in which the excretion of 17-ketosteroids has been measured by comparable methods are included in Table V. Of the total of 24 cases, 1 is an adult male, 16 are adult females, while 2 cases in male children and 4 in female children have been investigated. This distribution illustrates the great rarity of such tumors in adult males as compared with their incidence in adult females. The syndrome of basophilism almost always indicates the presence of an adrenal cortical tumor in children, frequently so in the adult woman, but only rarely so in adult men.

TABLE I: EXCRETION OF 17-KETOSTEROIDS IN THE URINE OF POSTPUBERTAL FEMALE PATIENTS

Pituitary basophilism			Adrenal virilism			Hirsutism		
Case no.	Age	17-Ketosteroid (mgm./day)	Case no.	Age	17-Ketosteroid (mgm./day)	Case no.	Age	17-Ketosteroid (mgm./day)
2		6.0	3		37.0	38	20	9.6
18		13.5	4		12.0	42		3.4
26		19.6	14		29.8	43		22.2
58	36	28.1	35	30	28.0	44	34	21.0
61		7.9	57		37.0	85	30	15.1
77		27.4	69		14.9	103	36	10.4
82	26	29.0	73		13.5	114	20	22.2
88	22	23.3	74		5.9	115	21	33.0
96	36	9.3	76		24.0	119	22	11.3
97	25	9.3	80		26.1	131	21	20.6
102	16	10.8	81	25	23.1	133	21	17.0
105	24	28.6	83		24.0	139	25	21.0
109	18	12.5	98		20.5	140	36	5.0
113	19	60.0	121		28.8			
120	34	15.1	124	36	22.8			
135	32	20.3	125	27	17.4			
141	20	37.0	126		22.7			
			127	28	18.9			
			130	19	13.3			
			137	19	27.3			

Case 99.—(A. B.) Woman aged 40. Admitted to hospital complaining of abdominal swelling, hirsutes, and increasing obesity. Estimation of 17-kestosteroids showed 126 mgm. per day. At operation a mass of tissue was removed, which, on section, proved to be a carcinoma of the adrenal cortex. The patient did well, but discharged herself, and no further investigation was possible.

In two of the cases described above (75 and 79) sufficient urine was obtained to make a chemical investigation of the 17-ketosteroids possible. An ether extract of the hydrolyzed urine was prepared and the neutral fraction of this extract separated. The neutral fraction was further divided into a ketonic and a non-ketonic fraction by the use of Girard's reagent "T". The hydroxy-ketones were separated from the ketonic

In considering the levels of excretion of 17-ketosteroids in these cases account must be taken of the patient's age and sex. There is general agreement in the literature that the average daily excretion of 17-ketosteroids in normal adults is about 13 mgm. for males and 8 mgm. for females. In children the excretion gradually increases up to the age of puberty. Talbot, Butler, and MacLachlan (13) give the average daily output in children as 1.3 mgm. (4 to 7 years), 4.0 mgm. (7 to 12 years), and 8.2 mgm. (12 to 15 years). When these standards are used as the basis of comparison it will be seen that, with two exceptions, all the excretions shown in Table V are relatively very high. One exceptional case, to which Callow and Crooke (3) drew attention, excreted only

[1] Specimens and sections of these tumors were demonstrated at a meeting of the Pathological Section of the Royal Society of Medicine, London, on November 17, 1942.

14.5 and 20 mgm. per day; that is, about twice the normal value. They suggest that the low figures might be accounted for by the patient's age and the fact that she was very ill. In the other exceptional case described by Fraser, Forbes, Albright, Sulkowitch, and Reifenstein (7), in which the excretion was 19.9 mgm. per day, the low figure may probably be attributed to the fact that the urine had been kept for 5 years at room temperature before the determination was made.

(b) *Cases of pituitary basophilism, adrenal virilism, or hirsutism, without adrenal tumor.*—The results obtained on postpubertal female patients showing these conditions are set out in Table I. Cases classified as pituitary basophilism, with the exception of No. 113, show a range of 6 to 37 mgm. per day, with a mean value of 18.6 mgm. In case 113 the daily excretion of 60 mgm. is very high. This value was obtained recently in a female patient, aged 19, in whom the chief feature was considerable obesity without signal hypertrichosis. Her weight at the time of investigation was 238 lbs. (108 k.). At the present time no further information is available, but the possibility of the presence of an adrenal tumor cannot be excluded. The 20 cases grouped under adrenal virilism show values very similar to those of the first category; the range is 5.9 to 37 mgm., with a mean of 22 mgm. The cases tentatively labeled hirsutism show a similar range (3.4 to 33 mgm.), with a rather lower mean of 16 mgm.

In general these results are very similar to the published values of other workers. Crooke and Callow (5), in 26 cases of virilism and one of basophilism without adrenal tumor, found outputs up to 35 mgm. per day. In 10 similar cases Callow and Crooke (3) found values of 7.5 to 30 mgm. In 67 postpubertal virilism cases Patterson, McPhee, and Greenwood reported values ranging from 6.4 to 33.4 mgm. per day, and similar results have been found by Talbot, Butler, and MacLachlan (13), and by Fraser, Forbes, Albright, Sulkowitch, and Reifenstein (7). Thus results already published together with those given in the present paper, which cover well over 150 cases, indicate that in virilism without adrenal tumor in postpubertal women excretions of 17-ketosteroids of up to 40 mgm. per day, or 5 times the normal amount, may be expected. Excretions in cases of proved tumor are usually well above this range, but an intermediate zone exists in which interpretation of the 17-ketosteroid determination is difficult.

(c) *Cases of pubertal virilism in female children.*—Of the 8 estimations (Table II) on prepubertal females, 2 are noteworthy. The very high values found in cases 1 and 70 are quite comparable with those found in cases of proved adrenal tumor. This is, again, in agreement with the findings of other investigators since Patterson, McPhee, and Greenwood (10) and Callow and Crooke (3) have reported very high excretions of 17-ketosteroids in cases of prepubertal virilism. Neither case 1 nor case 70 had an adrenal tumor. It has been suggested that a refinement of fundamental importance in the use of estimations of 17-ketosteroids as a diagnostic method for adrenal tumors would be the isolation of dehydro*iso*androsterone from the suspected urine, since this substance appears to be the characteristic compound responsible for the high values obtained in tumor cases. Support for this view was obtained in the present work.

From the urine of case 1, which has been fully reported by Richardson and Doll (11), relatively large amounts of androsterone were isolated. Dehydro*iso*androsterone was either absent, or present in an amount too small to be isolated by the procedure employed. Similar results were obtained on chemical examination of urine extracts from case 70. Thus it appears probable that prepubertal virilism is characterized by the excretion of abnormally large quantities of androsterone, in contrast with the high excretions of dehydro*iso*androsterone found in adrenal tumor cases.

TABLE II: EXCRETION OF 17-KETOSTEROIDS IN THE URINE OF FEMALE CHILDREN

Case no.	Age	17-Ketosteroids (mgm./day)	Remarks
1	7	90.0, 55.0, 125.0	Prepubertal virilism
13	9	30.4	Premature puberty
70	10½	140	Prepubertal virilism
101	13	19.8	Precocious sexual development
111	10½	21.0	Basophilism
116	9	8.4	Precocious menstruation
129	13	13.3	Virilism
136	6½	11.9, 6.6	Penis-like clitoris

(d) *High excretion of 17-ketosteroid without adrenal tumor or prepubertal virilism.*—Two cases of anomalously high excretion were encountered (cases 87 and 128, Table III).

Case 87.—In September, 1942, there was an onset of virilism. In December, 1942, the patient was admitted to the hospital in the later stage of pregnancy for removal of a dead fetus. The virilism was advanced, and an estimation of 17-ketosteroids was requested. This showed 158 mgm. per day. The fetus was found to be alive and a male child was born in January, 1943. Subsequent to the birth of the child the excretion fell to 23 mgm. per day. An operation was performed and an ovarian tumor removed. Postoperative estimation of 17-ketosteroids showed 12.8 mgm. per day. In view of the large fall in excretion that followed the birth of the child an estimation of 17-ketosteroids in the

child's urine, when he was about 3 weeks old, was made; 13.8 mgm. per liter were found. Control data for a child of this age are not available. Patterson, McPhee, and Greenwood (10) report that in an infant of 20 months the excretion was so low that it could not be accurately measured, but that it was less than 0.6 mgm. per liter. The value of 13.8 mgm. is, therefore, probably to be regarded as extremely high. No further information is available as yet about this case but an effort is being made to follow up both mother and child.

Case 128.—This patient had complained of swelling of the abdomen, increasing fatness of the face, hair growing on the chin, and mental dullness. A

TABLE III: EXCRETION OF 17-KETOSTEROIDS IN THE URINE OF FEMALE PATIENTS SUFFERING FROM VARIOUS DISORDERS

Case no.	Age	17-Ketosteroids (mgm./day)	Remarks
11	22	15.5	Homosexual
39	23	18.5	External characteristics entirely female but laparotomy revealed absence of tubes, broad ligaments, and uterus, and ovaries replaced by testis-like structures.
40	50	6.5	Carcinoma of breast
41	50	7.2	Carcinoma of breast
87	31	158.0	Virilism during pregnancy. Ovarian tumor removed.
91		9.1	Ovarian tumor
104	18	8.1	Sexual underdevelopment
128	38	80.0	Virilism. Large abdominal tumor present. Necropsy revealed carcinoma of splenic flexure of the colon.
132	30	20.4	Acromegalic
138	26	15.1	Early acromegaly
142	19	9.3	Obese pituitary dwarf

TABLE IV: EXCRETION OF 17-KETOSTEROIDS IN THE URINE OF MALE PATIENTS SUFFERING FROM VARIOUS DISORDERS

Case no.	Age	17-Ketosteroids (mgm./day)	Remarks
23	27	4.5	Undeveloped secondary sexual characteristics.
27	8	10.5, 6.4	Macrogenitosomia
28	10	9.3, 10.5	Gynecomastia
29	27	12.1	Unilateral castrate; second abdominal testis ?
30	18	11.3	Impotent
34		19.4, 16.5	Seminoma
78		28.6	Teratoma testis
90		32.2	Seminoma
92	14	35.9, 20.6	Precocious puberty
93	35	34.8	Impotent
95		15.4	Seminoma
100		20.0	Seminoma
107		10.3	Castrate
110	34	38.6	Acromegaly
112	32	13.1	Chorionepithelioma
117		33.0	Chorionepithelioma
122	34	33.2, 35.4	Basophilism
123	14½	14.3	Macrogenitosomia of the "juvenile hercules type"
134	21	17.8	Cachexia; pituitary in origin ?

TABLE V: REPORTED CASES OF ADRENAL CORTICAL TUMORS IN WHICH URINARY EXCRETION OF 17-KETOSTEROIDS HAS BEEN ESTIMATED

Sex	Age	17-Ketosteroids	Authors
M	25	40–64	Crooke and Callow, 1939 (5)
F	6	126–288	" " " "
F	—	45	Friedgood and Whidden, 1939 (8)
F	10	325	" " " "
F	3	160	Talbot, Butler, and MacLachlan, 1940 (13)
F	13	166	" " " " "
F	35	74	Fraser, Forbes, Albright, Sulkowitch, and Reifenstein, 1941 (7)
F	36	19.9	" " " " " " "
F	3½	176	" " " " " " "
M	1	28	Patterson, McPhee, and Greenwood, 1942 (10)
M	5	27	" " " " "
F	Adult	170	" " " " "
F	34	270	" " " " "
F	56	74	Talbot, Butler, and Berman, 1942 (12)
F	Adult	74 ?	" " " " "
F	25	215	Anderson, Hain, and Patterson, 1943 (1)
F	61	14.5, 20	Callow and Crooke, 1944 (3)
F	26	25–107	" " " "
F	41	76, 78	" " " "
F	38	54, 52	" " " "
F	54	83	Warren (1944)
F	42	690	" "
F	49	269	" "
F	40	126	" "

large, firm tumor, extending across the right hypochondrium and epigastrium, could be felt; 17-ketosteroid estimation showed 80 mgm. per day. The patient died 10 days after this estimation was made, and necropsy revealed an ulcerating and fungating carcinoma of the splenic flexure of the colon, which on microscopical examination was classified by the pathologist as a "trabecular polygonal-cell carcinoma." The suprarenals were almost unrecognizable, owing to postmortem decomposition, but no tumor was seen. No ovarian abnormality was found.

(e) *Miscellaneous cases.*—The results obtained in investigations of the remaining female patients (Table III) and of male patients (Table IV) call for little remark. No generalization can be made and no unexpected values were encountered.

SUMMARY

Urinary excretion of 17-ketosteroids was measured in the urines of 19 male and 73 female patients. In 4 cases of adrenal cortical tumor in adult females extremely high excretions were found; in 2 of these cases dehydro*iso*androsterone was isolated. Two cases of prepubertal virilism in girls also showed very high excretions, but isolation procedures showed the excess of androgen to be due to androsterone. A pregnant woman with virilism excreted large quantities of 17-ketosteroid; the child resulting from this pregnancy also showed an excessive excretion, but no explanation of these observations can yet be offered. About 10 times the normal excretion of 17-ketosteroids was observed in a patient suffering from carcinoma of the splenic flexure of the colon.

I am indebted to the clinicians who furnished the material for this investigation, and in particular to Professor F. Silberstein and Dr. Raymond Greene. My thanks are due also to The Sir Halley Stewart Trust for a Fellowship held during this period, and to The Anna Fuller Fund, and The British Empire Cancer Campaign for grants to this Institute.

REFERENCES

1. Anderson, A. F., Hain, A. M., and Patterson, J. A Case of Adrenal Carcinoma and Its Hormone Diagnosis. J. Path. & Bact., **55**:341-349. 1943.
2. Callow, N. H., Callow, R. K., and Emmens, C. W. Colorimetric Determination of Substances Containing the Grouping $-CH_2 \cdot CO-$ in Urine Extracts as an Indication of Androgen Content. Biochem. J., **32**:1312-1331. 1938.
3. Callow, N. H., and Crooke, A. C. Diagnosis of Adrenal Tumours. Estimation of 17-Ketosteroids in Urine. Lancet., **246**:464-465. 1944.
4. Crooke, A. C. A Change in the Basophil Cells of the Pituitary Gland Common to Conditions Which Exhibit the Syndrome Attributed to Basophil Adenoma. J. Path. & Bact., **41**:339-349. 1935.
5. Crooke, A. C., and Callow, R. K. The Differential Diagnosis of Forms of Basophilism (Cushing's Syndrome), Particularly by the Estimation of Urinary Androgens. Quart. J. Med., **8**:233-249. 1939.
6. Cushing, H. The Basophil Adenomas of the Pituitary Body and their Clinical Manifestations (Pituitary Basophilism). Buil. Johns Hopkins Hosp., **1**:137-195. 1932.
7. Fraser, R. W., Forbes, A. P., Albright, F., Sulkowitch, H., and Reifenstein, E. C. Colorimetric Study of 17-Ketosteroids in Urine; Survey of the Use of this Test in Endocrine Investigation, Diagnosis, and Therapy. J. Clin. Endocrinol., **1**:234-256. 1941.
8. Friedgood, H. B., and Whidden, H. L. The Assay of Crystalline and Urinary Androgens, with Special Reference to Their Measurement by a Colorimetric Method. New England J. M., **220**:736-741. 1939.
9. Kepler, E. J., Walters, W., and Piper, M. C. Tumors of the Adrenal Cortex: Diagnosis and Treatment and Report of a Case. Proc. Staff Meet., Mayo Clin., **13**: 353-361. 1938.
10. Patterson, J., McPhee, I. M., and Greenwood, A. W., 17-Ketosteroid Excretion in Adrenal Virilism. Brit. M. J., **1**:35-39. 1942.
11. Richardson, J. S., and Doll, W. R. S. The Juvenile Form of the Adreno-Genital Syndrome. Brit. M. J., **1**:501-502. 1939.
12. Talbot, N. B., Butler, A. M., and Berman, R. A. Adrenal Cortical Hyperplasia with Virilism: Diagnosis, Course and Treatment. J. Clin. Invest., **21**:559-570. 1942.
13. Talbot, N. B., Butler, A. M., and MacLachlan, E. A. Alpha and Beta Neutral Ketosteroids (Androgens). New England J. Med., **223**:369-373. 1940.

The American Association for Cancer Research, Inc.

Special Meeting of the Board of Directors

October 12, 1944

A special meeting of the Board of Directors was held in the office of the Acting Secretary-Treasurer, 333 Cedar Street, New Haven, Connecticut. The meeting was called by order of the Chairman, Dr. Shields Warren, who requested notices sent to all directors from the office of the Secretary-Treasurer at New Haven, Connecticut, and dated September 29, 1944. The meeting was called to order at 2:30 P. M. by the Chairman. Directors Howard B. Andervont, Halsey J. Bagg, James B. Murphy, George M. Smith, Shields Warren, and William H. Woglom were present. Reading of the minutes of the special meeting of the Board of Directors held February 12, 1943 and of the meeting of the Executive Committee held January 12, 1944 were waived, at the suggestion of the Chairman, by unanimous action. Minutes of these meetings were published in *Cancer Research,* 1943, Volume 3, pages 475-476, and 1944, Volume 4, pages 320-321, respectively.

NEW BUSINESS

It was voted that: "It be recommended that the Association request the Advisory Board of the journal, *Cancer Research,* to have a representative meet with the Directors of the Association at their next regular or special meeting to discuss the financial status of this publication."

It was voted that: "Tentative plans be made for the Thirty-Sixth Annual Meeting of the Members of the Association at a scientific session to be held during the coming year in conjunction with the Federation of the American Societies for Experimental Biology; the proposed meetings of the American Association for Cancer Research to be held on May 7th and 8th, 1945, in the city of Cleveland, Ohio."

Action Pertaining to Members

Resignations of the following five members were presented and accepted:

Arthur L. Amolsch, M.D., 1512 Antoine Street, Detroit, Mich.

Percival Bailey, Ph.D., M.D., University of Illinois, Chicago, Ill.

Herman R. Churchill, M.D., University of Pennsylvania, School of Dentistry, Philadelphia, Pa.

Leila Charlton Knox, M.D., St. Lukes Hospital, New York, N. Y.

Margaret R. Lewis, M.D., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

The Secretary presented the names of four members whose deaths had been made known to the office of the Association since the last meeting of the Executive Committee. It is with regret that the Association announces their loss.

Rigney J. D'Aunoy, M.D., 2936 Esplanade Avenue, New Orleans, La.

Georgine Luden, M.D., Ph.D., 1006 St. Charles Street, Victoria, B. C., Canada.

Edward W. Wallace, M.D., Ph.D., 1203 Ryland Avenue, Cincinnati, Ohio.

Reinhardt C. Wende, M.D., New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y.

The membership of two members of the Association was discontinued in accordance with action taken by the Board of Directors and as stipulated in the by-laws of the Association.

Smith Freeman, M.D., Ph.D., 643 Arlington Place, Chicago, Ill.

Fred C. Medinger, M.D., Memorial Hospital, New York, N. Y.

Nominations for membership of eleven applicants were presented and they were duly elected members in the association.

Joseph W. Beard, M.D., Department of Surgery, Duke University School of Medicine, Durham, N. C.

Helen M. Dyer, Ph.D., National Cancer Institute, Bethesda, Md.

Robert C. Horn, Jr., M.D., Department of Pathology, University of Pennsylvania, Philadelphia, Pa.

Charles Huggins, M.D., Department of Surgery, University of Chicago, Chicago, Ill.

Henry S. Kaplan, M.D., Department of Radiology, Yale University School of Medicine, New Haven, Conn.

Leo D. Moss, M.D., Olean General Hospital, Olean, N. Y.

Robert M. Perlman, M.D., National Cancer Institute, Bethesda, Md.

Jonathan E. Rhoads, M.D., Department of Surgery, University of Pennsylvania, Philadelphia, Pa.

Alfred Taylor, Ph.D., Department of Biology, University of Texas, Austin, Tex.

Lewis C. Scheffey, M.D., Department of Gynecology, Jefferson Medical College, Philadelphia, Pa.

Paul Swenson, M.D., Department of Radiology, Jefferson Hospital, Philadelphia, Pa.

Cancer Research

It was voted that: "The Secretary-Treasurer be authorized to pay to the journal, *Cancer Research,* a sum of $1.00 per member and an additional sum so that a total of $500.00 be contributed by the Association for the coming year, January 1, 1945 to December 31, 1945." This action continues the policy adopted at the meeting of the Board of Directors held in New York City on February 12, 1943.

Publications

It was voted that: "The Secretary be authorized to submit the proceedings of this meeting to be published in *Cancer Research* and to pay the expenses incurred by such publication."

Meeting adjourned 5:10 P. M.

Shields Warren, *Chairman, Board of Directors*
W. U. Gardner, *Acting Secretary-Treasurer*

Abstracts

Experimental Research, Animal Tumors

The Difference in Carcinogenicity between Shale Oil and Shale. BERENBLUM, I., and SCHOENTAL, R. [Oxford Univ. Res. Centre, Univ. of Oxford, Oxford, England.] *Brit. J. Exper. Path.*, **25**:95-96. 1944.

A chloroform extract of shale did not produce tumors when painted on mice. Hence the carcinogenic substances in shale oil are formed during the pyrolysis of the shale.—I. H.

Tumours of the Thyroid Produced by 2-Acetyl-Amino-Fluorene and Allyl-Thiourea. BIELSCHOWSKY, F. [Univ. of Sheffield, Sheffield, England] *Brit. J. Exper. Path.*, **25**:90-95. 1944.

Two series of rats, each consisting of 5 males and 5 females, received with the food (a) 2-acetyl-aminofluorene and allylthiourea and (b) allylthiourea only. All the thyroids in series (b) showed "uniform hypertrophy and hyperplasia with considerable loss of colloid." Nine out of 10 thyroids in series (a) showed in addition nodules, regarded as adenomas, of closely packed cuboid or cylindrical cells among which there might be spaces filled with colloid into which the surrounding cells proliferated in the form of papillomas. The larger nodules were from 0.5 to 1.5 mm. in diameter. In 3 females of series (a) larger nodules of less uniform structure were present, which are regarded as adenocarcinomas. The largest of these thyroids formed an irregular mass $10 \times 8 \times 5$ mm.; the new growth had penetrated the capsule at many points. Neither compound alone produces these neoplasms. References are given to other investigations in which adenomas developed in the thyroids of rats receiving a goiter-producing diet (Wegelin, Hellwig).—E. L. K.

The Fluorimetric Estimation of 3:4-Benzpyrene in Whole Mice. WEIL-MALHERBE, H. [Cancer Res. Lab., Royal Victoria Infirmary, Newcastle-on-Tyne, England] *Biochem. J.*, **38**:135-141. 1944.

Benzpyrene is added to a mouse carcase; after saponification the unsaponifiable fraction of the tissues is adsorbed and eluted repeatedly, and the fluorescence transmitted by filters at 400 mμ is estimated photoelectrically by comparison with that from a standard solution of quinine sulfate. The original paper should be consulted for the theory of the adsorption process, the purification of reagents, and all technical details. "The limit of sensitivity is determined by the blank, i.e., the fluorescence intensity of extracts from benzpyrene-free mice. The average value of 54 experiments was found to be 0.50 μg. in terms of benzpyrene. . . . . The mean recovery of 36 experiments ranging from 3 to 300 μg. benzpyrene was found to be 87.36 per cent. . . . . The standard error of the same recovery experiments, after correction for blank and losses, varied between 2.3 and 5 per cent. It rose to 25.5 per cent. for 0.5 μg., as compared with a standard error of 23.4 per cent. to be expected on the basis of the standard deviation of the blank."—I. H.

A Note on the Fluorimetric Estimation of 3:4-Benzpyrene by the Method of Miller and Baumann. WEIL-MALHERBE, H. [Cancer Res. Lab., Royal Victoria Infirmary, Newcastle-on-Tyne, England] *Biochem. J.*, **38**:141. 1944.

O_2 quenches fluorescence of hydrocarbons much more than the fluorescence of extracts of tissues. "If the quenching ratios of both are known, the composition of a mixture can be deduced from the increment of fluorescence obtained on changing from an oxygenated to an oxygen-free medium." This method (MILLER, J. A., and BAUMANN, C. A. *Cancer Research*, **3**:849. 1943) is satisfactory for benzpyrene added to extracts of mouse tissue provided they are purified by adsorption (see preceding abstract). It is quite unreliable for crude extracts such as those employed by Miller and Baumann.—I. H.

Tissue Nucleic Acids. 1. Ribonucleic Acids and Nucleotides in Embryonic and Adult Tissue. DAVIDSON, J. N., and WAYMOUTH, C. [Univ. of Aberdeen, Scotland] *Biochem. J.*, **38**:39-50. 1944.

Data are given for the water content and nucleoprotein phosphorus of the heart, liver, brain, nerve, and muscle, and Rous sarcoma of the fowl. The tumor contains more water (88.2%) and nucleoprotein phosphorus (516 mgm. per 100 gm. dry weight) than do the other tissues.—E. L. K.

Esterase Activity of Blood Serum of Four Strains of Mice. SHIMKIN, M. B., GREENSTEIN, J. P., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:29-30. 1944.

The esterase activity of the serum of mice from various inbred strains was determined, methyl butyrate being used as substrate. Strains C57, I, and C were studied, also mice with and without tumors from strain C3H, and descendents of C foster-nursed by C3H mothers. The esterase activity of the sera from the various strains differed considerably, but no relation was found between the serum esterase activity and the tendency to develop spontaneous mammary tumors.—H. Q. W.

Esterase (Butyric Esterase) Activity of Normal and Neoplastic Tissues of the Mouse. GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:31-34. 1944.

The esterase of numerous normal and neoplastic tissues was determined. The extracts of the methyl butyrate tissues were diluted until a linear relationship between esterase activity and concentration was obtained. The normal tissues, in order of decreasing esterase activity, were: pancreas, intestinal mucosa, liver, kidney or spleen,

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington D. C

lung, gastric mucosa, lymph nodes, heart or skeletal muscle, brain, thymus or skin. The neoplastic tissues examined included 3 hepatomas, 2 melanomas, tumors of mammary gland, lung, salivary gland, stomach, and intestine, 2 sarcomas, and a lymphoma. Except for the hepatomas, the esterase activity of all the tumors was low. In all cases in which comparison was possible the esterase activity of the tumors was less than that of the tissue of origin, but the difference was less for the hepatomas than for most other tumors.—H. Q. W.

Comparative Oxidase Activity of Melanotic and Amelanotic Melanomas. GREENSTEIN, J. P., and ALGIRE, G. H. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:35-38. 1944.

The melanotic and amelanotic regions of the transplanted mouse melanoma S 91 (in the C and dba strains of mice) as well as the transplanted Harding-Passey pigmented melanoma (in C strain mice) were examined for tyrosinase, dopa oxidase, and cytochrome oxidase activity in presence and absence of cyanide. Sand-ground tissue, homogenized and suspended in phosphate buffer, was employed as the source of enzyme activity; the oxygen uptake in the presence of the various substrates was measured in the Warburg manometer. The melanotic tumor tissues showed considerable tyrosinase and dopa oxidase activity that could be largely poisoned by cyanide; whereas the amelanotic tissue was practically devoid of this type of enzymatic activity. There was only a slight, if any, difference between the two types of tumor tissue with regard to cytochrome oxidase activity. The amelanotic melanoma, furthermore, lacked the cyanide-insensitive system of the pigmented tumor tissues capable of oxidizing *p*-phenylenediamine with molecular oxygen. More recent experiments on a deeply pigmented human melanoma and an amelanotic human tumor yielded results similar to those reported here for mouse tumors of this type.—K. G. S.

Note on the Cystine Oxidase Activity in Normal and Neoplastic Tissues of the Mouse. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:39-40. 1944.

Cystine oxidase is an enzyme distinct from amino acid oxidase. It catalyzes the degradation of cystine or cysteine under either aerobic or anaerobic conditions. The enzyme was found to be present in the liver, kidney, and pancreas of the mouse, the activity decreasing in the organs in the order in which they are listed. It was completely absent from numerous other normal tissues examined. It was also absent from 15 different neoplastic tissues, including 3 hepatomas.—H. Q. W.

Chemical Studies on Human Cancer. I. Cytochrome Oxidase, Cytochrome *C*, and Copper in Normal and Neoplastic Tissues. GREENSTEIN, J. P., WERNE, J., ESCHENBRENNER, A. B., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**: 55-76. 1944.

The work was made possible by the co-operation of the National Cancer Institute with the Memorial Hospital, Cornell University Medical College, and New York Hospital. Eighty normal human tissues of various types and 60 human neoplasms were examined for cytochrome oxidase activity (manometric measurements of rate of oxygen uptake of ground tissue suspensions with excess cytochrome *c* and *p*-phenylenediamine), for cytochrome *c* content (manometric measurements of oxygen uptake by isolated cytochrome *c* with standardized veal heart cytochrome oxidase preparations), and in some instances, for copper content (colorimetric diethyldithiocarbamate method). The rate of oxygen consumption of the tissue suspensions was limited by the cytochrome *c* concentration; cytochrome oxidase was always present in excess. The assumed respiration of heart, skeletal, and diaphragm muscle, liver, kidney, and brain tissue may be accounted for in terms of the cytochrome oxidase-cytochrome *c* system. The cytochrome system of all other tissues, and especially that of malignant tumors, on the other hand, accounted for but a small fraction of the respiration of these tissues in intact form. On the whole, the range of activity of cytochrome oxidase and of cytochrome *c* content was much narrower among all the neoplastic tissues than among all normal tissues. Human tissues exhibited lower cytochrome oxidase activity than did the tissues of the various animal species examined. Liver had the highest copper content of all tissues studied. The copper concentration of all neoplasms studied was of the same order of magnitude, while there were considerable variations in copper content among the normal tissues. Pigmented and nonpigmented melanomas possessed nearly the same cytochrome oxidase activity. Pigmented melanomas, in addition, possessed cyanide-sensitive systems capable of oxidizing tyrosine and dihydroxyphenylalanine to melanin, and cyanide-insensitive systems attacking *p*-phenylenediamine.—K. G. S.

Effect of a Low Lysine Diet on Mammary-Tumor Formation in Strain C3H Mice. WHITE, F. R., and WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:41-42. 1944.

Previous work showed that virgin female mice of the C3H strain developed no mammary tumors when fed a diet low in cystine, although adequately fed controls had a mammary tumor incidence of 97.4% at an average age of 10.4 months. The experimental animals probably did not show estrus. In the present work C3H virgin female mice were fed a diet low in lysine. Twenty-five per cent of the animals developed mammary tumors at an average age of 12.9 months. The appearance of the mammary glands and ovaries showed that estrus occurred occasionally. It appears that the effect of deficient diets in suppressing the development of spontaneous mammary tumors is due to the suppression of estrus.—H. Q. W.

Effect of Caloric Restriction on Mammary-Tumor Formation in Strain C3H Mice and on the Response of Strain dba to Painting with Methylcholanthrene. WHITE, F. R., WHITE, J., MIDER, G. B., KELLY, M. G., and HESTON, W. H. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:43-48. 1944.

A 50% reduction in calories, without alteration in the amount of dietary essentials, reduced the incidence of mammary gland tumors from 100% to 12.5% and 18.2% in strain C3H virgin and breeding females, respectively. In methylcholanthrene painted strain dba mice, 50% caloric restriction caused a reduction in the development

of leukemia from 96.2% to 35%, and an extension of the mean latent period from 73 days to 237. Twenty five per cent of the calorie-restricted animals, although they did not develop leukemia, did develop other neoplasms. Vaginal smears of the calorie-restricted C3H virgin females showed no evidence of estrus. The effect of dietary restriction on estrus in the other groups of animals is not reported.—H. Q. W.

Effect of Diet upon the Development of Mammary Glands in Strain C3H Mice. WHITE, F. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:49-53. 1944.

Gross mounts were prepared of the mammary glands of C3H female mice that had been kept under various dietary conditions. In virgin females kept on a low cystine diet from weaning time there was no growth of mammary tissue; on a low calorie diet there was little growth; on a low lysine diet there was variable retardation of growth. Continuous estrus induced by stilbestrol caused growth of mammary tissue in animals on a low cystine diet. Breeding females put on a low calorie diet at 4 to 6 months of age later showed considerable atrophy of mammary tissue.—H. Q. W.

The Effect of Chemical Carcinogens on Virus-Induced Rabbit Papillomas. ROUS, P., and FRIEDEWALD, W. F. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **79**:511-537. 1944.

The authors found that the application of tar and methylcholanthrene to virus-induced papillomas caused them to become carcinomatous with great rapidity. These cancers were of the same types that arise spontaneously from the application of the carcinogens alone, but appeared in a much shorter time. The evidence of the experiments would seem to indicate that the production of the cancers was due to changes in the environment of the virus brought about by the carcinogens rather than due to an ordinary stimulation of cell proliferation. The relationships between virus and chemical carcinogens are discussed.—D. S.

The Recoverability of Virus from Papillomas Produced Therewith in Domestic Rabbits. FRIEDEWALD, W. F., and KIDD, J. G. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **79**:591-605. 1944.

While the papilloma virus is readily recoverable from most cottontail rabbit papillomas, the virus cannot ordinarily be obtained from papillomas of domestic rabbits. However, these authors find that, if the skin of domestic rabbits is first rendered hyperplastic before the injection of the test extracts of domestic rabbit papillomas, the presence of virus can be demonstrated. The yield of virus is influenced by the virus strain and the rabbit host and is small in comparison with the amount obtained from cottontail rabbit papillomas. Also, it is shown that the demonstrable amount of infective virus in both the papillomas of domestic and cottontail rabbits is approximately proportional to the amount of specific antibodies produced by these extracts. The results indicate that much less virus exists in infective or antigenic form in the papillomas of domestic rabbits than in those of cottontail rabbits.—D. S.

Induction of Leukemia in Mice. KIRSCHBAUM, A., and KAPLAN, H. S. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Science*, **100**:360-361. 1944.

Young adult mice of strains F, A, dba_{212}, and dba_{12} were exposed to x-rays (80 r per day for 9 to 11 successive days), or to methylcholanthrene (0.5% solution in benzene given percutaneously twice weekly), and the development of leukemia studied.

Mice of the F, dba_{212}, and dba_{12} strains (with high, medium, and low incidences of spontaneous leukemia respectively) were all susceptible to induction of leukemia with methylcholanthrene, but resistant to x-rays. Among mice of the A strain, with a low incidence of spontaneous leukemia, the reverse was true, *i.e.* methylcholanthrene had no effect, but x-raying produced leukemia.—R. B.

Tissue Changes Produced in C3H Mice by 50 r Whole Body Exposure. NETTLESHIP, A. [U. S. Public Health Service] *Radiology*, **42**:64-70. 1944.

Two hundred kv. radiation with 0.5 mm. Cu filtration was used in exposing mice to 50 r radiation. Early examination showed widespread changes, most of the cells surviving and returning quickly to normal.—R. E. S.

Radioactivity and Lung Cancer; A Critical Review of Lung Cancer in the Miners of Schneeberg and Joachimsthal. LORENZ, E. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:1-15. 1944.

A review of clinical and experimental reports published by various European authors on the high incidence of lung cancer (mainly squamous cell carcinoma), when considered in the light of present knowledge on the tolerance of different radon (radium emanation) concentrations in the air, leads to the conclusion that "The opinion that radon is the sole cause of the lung cancer of the miners . . . cannot be maintained." Pneumoconiosis produced by the dust in the mines, chronic irritation caused by respiratory diseases, arsenic, radioactive substances, and possibly a hereditary susceptibility to the disease are considered as potentially contributing factors.—K. G. S.

Recognition of Liver Cirrhosis Induced by Paradimethylaminoazobenzene (Butter Yellow) by X-ray and Thorium Dioxide. STEINBERG, B., WALLIKER, C. T., and MARTIN, R. A. [Toledo Hosp. Inst. of Med. Research, Toledo, Ohio] *Proc. Soc. Exper. Biol. & Med.*, **55**:165-167. 1944.

Albino rats were given intracardially 0.5 cc. of thorium dioxide sol (thorotrast). A similar dose may be given every 2 to 3 months for prolonged periods. The thorotrast is deposited principally in the sinusoids of the liver and capillaries of the spleen, and these organs can be visualized by roentgenogram. Hematoxylin and eosin preparations of cirrhotic liver tissue of rats fed *p*-dimethylaminoazobenzene showed that the thorotrast was concentrated in the connective tissue and was intracellular. The thorotrast was markedly absent, or present only in minute quantities, in liver areas showing duct hyperplasia, duct adenocarcinoma, hepatoma, and adenocarcinoma of liver cells. Correlation of microscopic preparations and the roentgenograms indicated that the visible radiopaque lines outlined connective tissue, which replaced liver cells and distorted the organ architecture. The nonvisualized areas represented more or less intact liver tissue. Evaluation of visualized and nonvisualized areas established the degree of damage and scarring. In the spleen, the thorotrast was lodged mainly in the capillaries of the pulp,

with occasional small accumulations of the chemical in the periphery of the follicles. Visualization of the spleen allowed determination of its size.—M. B.

Development of Tumors in the Rat Ovary After Transplantation into the Spleen. Biskind, M. S., and Biskind, G. S. [Beth Israel Hosp., New York, N. Y., and Mt. Zion Hosp. and Univ. of California Med. Sch., San Francisco, Calif.] *Proc. Soc. Exper. Biol. & Med.*, **55**:176-179. 1944.

A number of adult female rats of the Sherman strain were castrated, and one ovary was transplanted to the spleen. After about 2 weeks, rats showing estrous reactions were discarded. The others were kept on a complete diet for about 11 months thereafter. At the end of this time 9 animals remained; of these, 6 were completely anestrous, and 3 gave smears showing an occasional epithelial or squamous cell but never a complete estrous smear.

Microscopic sections were made of the vagina and uterus, pituitary body, and the spleen containing the transplanted ovary. In every case the ovarian mass in the spleen was considerably enlarged. Histologically, all transplanted ovaries were greatly hypertrophied and composed of masses of theca cells. Three large tumors were found, apparently of the granulosa cell type, and in 2 other tumors there were smaller nodules of the same cellular structure. The uterus, vagina, and pituitary body showed the type of atrophy associated with castration.—M. B.

Viability of a Transmissible Fowl Tumor (Olson) upon Storage at Low Temperatures. Burmester, B. R., Brandly, C. A., and Prickett, C. O. [Regional Poultry Research Lab., U. S. Dept. Agriculture, East Lansing, Mich.] *Proc. Soc. Exper. Biol. & Med.*, **55**:203-204. 1944.

Samples of transmissible fowl lymphoid tumor grown in pectoral muscle were excised aseptically, minced, and placed in pyrex glass or lusteroid tubes. The samples were frozen by immersing the tubes in 95% ethyl alcohol and lowering the temperature between 0° C. and −20° C. by adding small pieces of solid CO_2. After freezing, the tubes were kept at −65 to −75° C. Tumor samples were thawed under running tap water just prior to inoculation. One cubic centimeter of tumor suspension in Tyrode solution, saline, or heavy mineral oil was injected into the pectoral muscle. No significant reduction or change was found in the capacity of the tumor to produce typical growths upon inoculation.—M. B.

Absence of Daily Rhythm of Growth of Malignant Neoplasms. Dublin, W. B. [Western State Hosp., Ft. Steilacoom, Wash.] *Northwest. Med.*, **43**:232. 1944.

Mitotic figures were counted in biopsy specimens of 9 malignant tumors. Six specimens were obtained from each tumor at 4 hour intervals. No rhythm of mitosis, such as has been demonstrated for normal tissues, was apparent.—E. E. S.

"Folic Acid" a Tumor Growth Inhibitor. Leuchtenberger, C., Lewisohn, R., Laszlo, D., and Leuchtenberger, R. [Mt. Sinai Hosp., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **55**:204-205. 1944.

Intravenous injections of 0.16γ "folic acid concentrate" and 0.025γ *L. casei* factor inhibited tumor growth significantly in 7 mice. Similar results were obtained with "folic acid concentrate" in 6 experiments on 117 mice, and with *L. casei* factor in 10 experiments on 364 mice. On a weight basis, the *L. casei* factor was many hundred times stronger than inositol.

Female Rockland mice inoculated with sarcoma 180 were used.—M. B.

The Effect of Mercury-Indigo-Disulphonate on Liver Cancer of Rats. Davis, J. E. [Chicago, Ill.] *Canad. M. A. J.*, **50**:553-554. 1944.

The oral administration proved more effective for liver cancer of rats than for mammary cancer of mice. The compound was unable to prevent cancer of the liver but seemed to help towards its cure.—M. E. H.

Feminization in Male Dogs. A Syndrome Associated with Carcinoma of the Testis and Mimicked by the Administration of Estrogens. Mulligan, R. M. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Am. J. Path.*, **20**:865-875. 1944.

An analysis is given of feminization in 6 cases of adenocarcinoma of the testis of dogs, collected from the literature. Protocols and discussion of experiments in which dogs were given stilbestrol and other estrogens are also presented.—J. G. K.

Some Statistical Aspects of Canine Tumors. Mulligan, R. M. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Arch. Path.*, **38**:115-120. 1944.

General review.—J. G. K.

Seminoma in the Testis of a Dog. Hueper, W. C. [Warner Inst. for Therapeutic Research, New York, N. Y.] *Am. J. Clin. Path.*, **14**:418-421. 1944.

A testicular seminoma of the sertolian type which occurred in one of 130 male dogs studied is described. Attention is called to the fact that canine seminomas can be traced to the seminiferous epithelium and are thus not of teratomatous origin.—Author's summary.

Spontaneous and Transplanted Reticulum Cell Sarcomas in Wistar Rats. Farris, E. J., and Yeakel, E. H. [Wistar Inst., Philadelphia, Pa.] *Am. J. Path.*, **20**:773-781. 1944.

Reticulum cell sarcomas developed in 9 animals of a group of 1,000 Wistar albino rats, and similar growths appeared in 5 of 185 gray Norway rats. The growths are described and illustrated, and their successful transplantation is reported.—J. G. K.

Clinical and Pathological Reports

Diagnosis—General

Use of B-Proteus OX19 Agglutination in Diagnosis of Cancer. Oppenheim, A., and Warriner, R. [Memorial Hosp. for the Treatment of Cancer and Allied Dis., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **55**:190. 1944.

In a previous study it appeared that the sera of women with malignant neoplastic disease strongly agglutinated proteus organisms, and it was thought this agglutina-

tion test, though non-specific, often might yield evidence for the presence of cancer. The titer of *B. proteus* OX19 agglutinins was measured in the sera of 30 normal women, 27 with mammary carcinoma, and 3 with carcinoma of the cervix. The diagnosis of carcinoma in each case was confirmed by biopsy. With the methods used, no difference was shown by the 3 groups.—M. B.

Feather Germ Reaction to Urine from Patients with Cancer and Other Conditions. A Preliminary Study. JUHN, M. [Maryland Agric. Exper. Station, Univ. of Maryland, College Park, Md.] *Arch. Path.*, **37**:383-386. 1944.

The pigmentation reaction of the growing feather germ of the Brown Leghorn capon was used to record the effects of injected urines from cancerous, other pathologic, and normal subjects. Positive and negative reactions were obtained from various individuals in all 3 groups.—J. G. K.

THERAPY—GENERAL

One Year Observations of the Treatment of Cancer with Avidin (Egg White). KAPLAN, I. I. [Bellevue Hosp. and New York Univ. Coll. of Med., New York, N. Y.] *Am. J. M. Sc.*, **207**:733-743. 1944.

The author states that 10 patients having malignant growths of various kinds, as proved by pathologic examinations, were chosen for study and treatment. Three of the patients were given avidin therapy alone, while in the others roentgen ray therapy or surgery was employed in addition to the egg white treatment.

Two cases (one a carcinoma of the stomach, and the other a carcinoma of the breast with metastasis) are only mentioned; brief details are given of the remaining 8 patients, of whom 1 (with carcinoma of the lung) died 10 months after egg white and roentgen ray treatment had been instituted. The rest seemed to show some improvement in "general condition." In 1 patient (with carcinoma of the tongue) there seemed to be some recession in the size of the primary growth, though the regional nodes did not change in size; in another patient (with metastatic squamous cell carcinoma in the neck), given concentrated avidin in amounts up to 2,000 units per day during 4 months, the tumor finally increased in size and ulcerated through the skin. There was no effect on the leukocyte level in the 2 cases of leukemia.

Some of the patients received a total of more than 12,000 egg whites; none developed signs of biotin deficiency.

The author states in conclusion that "as yet no definite cure of cancer has been brought about through the use of egg white therapy . . . in a selected group of cases." He feels, however, that the avidin therapy may have played a beneficial role in some of the cases, and that the method of treatment might be improved if a definite biotin deficiency could be induced. He makes no mention of the fact that certain transplanted tumors will grow readily in animal hosts that manifest severe induced biotin deficiency, as shown by West and Woglom (*Cancer Research*, **2**:324-331. 1942).—J. G. K.

Control Factors in the Surgery of Cancer Patients. SUGARBAKER, E. D. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *J. Missouri M. A.*, **41**:112-115. 1944.

Cancer patients in general are poor operative risks. A thorough evaluation of the patient's clinical status should include the potentialities (pathologic, radiotherapeutic, and surgical) of the tumor itself. Emphasis during the postoperative period should be on the prophylaxis of complications, mainly atelectasis, ileus, and shock.—M. E. H.

BREAST

Cause of Mammary Carcinoma. PYBUS, F. C. [J. H. Burn Lab., Royal Victoria Infirmary, Newcastle-on-Tyne, England] LETTERS TO THE EDITOR. *Lancet*, **247**:360. 1944.

The author is investigating the Bittner factor by feeding mice of a low mammary cancer strain on human mammary carcinoma, and he asks for milk from any woman who has, or has had, mammary carcinoma for similar feeding experiments.—E. L. K.

Breast Tumors and Clinical Differentiation. KUNZ, G., JR. [Tacoma, Wash.] *Northwest. Med.*, **43**:101-104. 1944.

Brief mention is made of certain benign tumors, and there is a more extensive discussion of the etiology or diagnostic features of chronic mastitis, fibroadenomas, cystic disease, papillomas, tuberculosis, Paget's disease, sarcoma, and carcinoma. Hereditary predisposition to breast carcinoma is noted.—E. E. S.

Surgery of Breast Tumors. RITCHIE, C. B. [Tacoma, Wash.] *Northwest Med.*, **43**:106-107. 1944.

It is stated that proof is lacking that earlier and more radical breast amputations are obtaining a commensurate longevity for the patient. It is suggested that many patients need only be subjected to a simple mastectomy. Mention is made of various series of surgically treated cases, but the actual references are not given. The author believes that preoperative and postoperative irradiation alters the survival rate very little. The conclusion is reached that until better procedures are developed, the present axillary dissection is still the best radical operation even though paths of spread of the cancer, other than through the axilla, are not checked.—E. E. S.

Operability Versus Curability of Cancer of the Breast. PORTMANN, U. V. [Cleveland, Ohio] *Ohio State M. J.*, **40**:742-745. 1944.

The difficulty in evaluating reports on various modes of therapy, when there is such great diversity in the type and extent of the tumor being treated, is discussed at length. A classification of breast cancer based on clinical and pathological criteria is offered, and an analysis of 1,022 unselected cases is used to illustrate the applicability of this classification. The conclusion is reached that irradiation therapy is preferable to radical mastectomy for patients with one or more clinical manifestations listed as criteria of incurability.—E. E. S.

I. Fore-Quarter Amputation for Carcinoma of the Axillary Tail of the Breast. II. Carcinoma. ? Metastasis or New Growth. III. Radium Necrosis Following Old Case of Carcinoma of Breast. FITZWILLIAMS, D. C. L. *Proc. Roy. Soc. Med.*, **37**:497. 1944.

Descriptions of cases.—E. L. K.

True Hermaphroditism. Report of a Case with Mammary Carcinoma. MORIARTY, J. D. [New Hampshire State Hosp., Concord, N. H.] *Am. J. Path.*, **20**:799-807. 1944.

In an aged patient with senile psychosis, true hermaphroditism was confirmed by the demonstration of a right ovotestis and left scrotal testis. Secondary sexual characteristics were of feminine type. In the left breast there was a primary adenocarcinoma.—J. G. K.

Female Genital Tract

Hemorrhagic Tumors of the Ovary. Brookbank, E. B. [Seattle, Wash.] *Northwest. Med.*, **43**:170-171. 1944.

After a brief mention of ovarian tumors in general, the author discusses the etiology, classification, symptoms, diagnostic features, and treatment of endometriosis of the ovary and other organs. For therapy of endometriosis, resection is recommended except in widespread involvement of the rectum and rectovaginal septum where roentgen therapy is regarded as the safer procedure.—E. E. S.

Necrotic Uterine Fibromyoma Complicating Pregnancy. Case Report. Schneider, N. A. [St. Anthony's Hosp., St. Louis, Mo.] *J. Missouri M. A.*, **41**:164-165. 1944.

A case report. Necrosis or acute degeneration of a uterine fibromyoma during pregnancy is rare.—M. E. H.

Carcinoma of the Cervix Coincident with Pregnancy. Maino, C. R., and Mussey, R. D. [Mayo Foundation and Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **47**:229-244. 1944.

Of 3,570 cases of carcinoma of the cervix seen at the Mayo Clinic during a period of 32 years, pregnancy was present in 26. The average number of previous pregnancies was 6, and the average number of children was 4. The number of pregnancies did not affect the prognosis. When the carcinoma was diagnosed in the later months of pregnancy, the lesion was well advanced and the prognosis poor. Pregnancy may occur after carcinoma of the cervix has developed. A family history of carcinoma was elicited in 7 of 20 cases of pregnancy seen before 1941, the type of carcinoma in relatives not being stated. Bleeding was the initial symptom in most cases; in 25% of the cases the patient did not know she was pregnant.

In contrast to the preferred treatment for non-pregnant patients, operation is felt to be the treatment of choice if the lesion is operable, with supplementary irradiation therapy. If the extent of the lesion permits operation and the fetus is not yet viable, total hysterectomy followed by irradiation is recommended. If the lesion is operable and the fetus viable, the procedure is cesarean section followed by panhysterectomy and postoperative irradiation. When the lesion is non-operable and the fetus viable, cesarean section is followed by irradiation; in cases in which the fetus is not yet viable, sufficient irradiation is employed to treat the tumor and incidentally abortion occurs.

In cases in which the lesion was operable, total abdominal hysterectomy produced the best results. In 57% of the cases where this procedure was employed patients were free of recurrences 5 years after operation.

The prognosis seemed to be at least as favorable in carcinoma of the cervix associated with pregnancy as in those cases not thus associated.—A. K.

Cancer of the Cervix in Pregnancy. Morse, A. H. [New Haven Hosp., New Haven, Conn.] *Connecticut M. J.*, **8**: 592-595. 1944.

Report of 3 cases. The lesions were treated with x-ray and radium.—M. E. H.

Local Eosinophilia in Malignant Neoplasms. Gill, A. J. [Univ. of Tennessee, Memphis, Tenn.] *J. Lab. & Clin. Med.*, **29**:820-824. 1944.

Local eosinophilia was observed in 7.7% of a series of 309 cases of epidermoid carcinoma of the cervix. The possible meaning of the finding is discussed.—J. G. K.

Discussion on Chronic Vulval Skin Lesions. Bonney, V., Brain, R. T., Savill, A., Wright, S. W., Bottomley, J., and Semon, H. C. *Proc. Roy. Soc. Med.*, **37**: 431-434. 1944.

Bonney has observed that leukoplakia precedes almost all cases of carcinoma of the vulva.

The discussion related essentially to the differential diagnosis of a number of causes of vulval pruritus. These included leukoplakic vulvitis, lichen planus, lichen sclerosus, lepidosis vulvae, kraurosis, vulval psoriasis, various types of intertrigo, localized scleroderma, vitiligo, and monilia infections. Treatment was by pelvic diathermy, by estrogenic therapy, and by radiotherapy, combined with dermatological measures and relief of concomitant *B. coli* infection.—L. W. P.

Gastrointestinal Tract

Secondary Carcinoma of the Esophagus as a Cause of Dysphagia. Toreson, W. E. [McGill Univ., Montreal, Canada.] *Arch. Path.*, **38**:82-84. 1944.

An analysis of 26 cases.—J. G. K.

Achalasia of the Esophagus. Niehaus, R. F. [State Univ. of Iowa Coll. of Med., Iowa City, Iowa] *J. Iowa M. Soc.*, **34**:145-148. 1944.

The differential diagnosis of achalasia, carcinoma of the esophagus or of the fundus of the stomach, and Zenker's diverticulum is discussed. Proved malignant neoplasm was discovered in 5 instances in this series of 40 patients, although after roentgen examination and esophagoscopy the condition had been diagnosed as achalasia. This represents an error of approximately 13%. In these cases, carcinoma is usually suspected, although diagnosis is difficult.—M. E. H.

Sarcoma of the Stomach. Porritt, A. E., Hughes, K. E. A., and Campbell, R. J. C. *Brit. J. Surg.*, **31**:395-398. 1944.

Report of a case in a male aged 27. The tumor is described as a round-cell sarcoma, but its origin is indefinite. The radiological appearances are said to be characteristic—a filling defect with regular contours, a normal or exaggerated mucosal pattern, and persistence of peristalsis.—L. W. P.

Duodenal Carcinoma. Burke, E., Perkel, L. L., and Gnassi, A. M. [Jersey City Med. Center, Jersey City, N. J.] *Am. J. Surg.*, **62**:267-271. 1943.

Case report.—W. A. B.

Book Reviews

ADVANCES IN ENZYMOLOGY AND RELATED SUBJECTS OF BIOCHEMISTRY. Volume IV. Edited by F. F. Nord, and C. H. Werkman. New York: Interscience Publishers, Inc. 1944, viii + 332 pages. Price $5.50.

This is the fourth volume of the valuable series of review articles collected by these editors and in general the same high level of presentation has been maintained. This kind of abbreviated monograph is of particular value to the biochemist in the field of cancer research, for in few other border-line subjects is it necessary to possess so wide a command of the biochemical literature. Although the biochemical oncologist is often compelled to ride on the pathologist's coattails (and a good thing, too) he must be, at the least, an independent entity within his own subdivision of the field. The *Advances in Enzymology,* the *Annual Review of Biochemistry,* and similar compilations assist him in this respect. The reader is warned, however, that much of the information in these books is predigested for him, and different authorities may vary considerably in their degree of mental achlorhydria. In places where the book reviewer and the original author do not agree, it may be that the digestion of the former is more at fault than that of the latter and the reader is left in a quandary; in cases of doubt the obvious solution is for the reader to go back to the original literature and judge for himself. In any event he is the gainer.

The various reviews in the volume under consideration are discussed here in the order of their appearance.

Addison Gulick has contributed a review entitled "The Chemical Formulation of Gene Structure and Gene Action." The second half of this review, which embodies chiefly the author's own views, is interesting and thoughtful. It is all the more unfortunate, therefore, that so many errors have crept into the first half. The author's discussion of proteins is particularly open to question. For example, the presence of long polypeptide chains has not been proved but only inferred, not in native but in denatured protein molecules. The so-called Svedberg series of molecular weights, whereby protein molecules are supposedly built up of fundamental units of weight 16,000 to 17,000, has been abandoned. The Svedberg unit is now commonly used by protein chemists to refer to the sedimentation constant $\times 10^{13}$. The protein constituents of viruses are not all "giant molecules" but may vary considerably in size, and there is no proof that they are "especially provided with the highly basic amino acid moieties such as are found in chromosomes." The length of the nucleic acid molecule of the tobacco mosaic virus nucleoprotein is given as 3,000 A and it is stated that "this inflexible structure" equals or exceeds the "standard virus particle" in length. There is further no reason to suppose that the structure of any molecule, save that of the benzene ring, is "inflexible." There is no evidence to support the enol structure of the peptide bond given on page 9, nor the statement that "nucleic acid is largely non-specific, varying little from organism to organism." The following statement is obscure: "amino acids that belong exclusively to the *levo*-series as classified by the rotation of the crucial carbon . . . . with *d* and *l* understood in this way, no amino acid of the *d* series is physiological." The *levo* and *dextro* series of amino acids are derived on the basis of the direction of rotation of the parent lactic acids and have nothing to do with the property of the asymmetric—not "crucial"—alpha carbon of the amino acids. Furthermore, as Hotchkiss points out later in the same volume, many microorganisms possess *dextro* amino acids quite naturally or physiologically. There is no evidence whatever for the hypothesis of the participation of aldehydes in protein synthesis nor that "amino acids would have to surrender oxygen to some substance undergoing oxidation before they become fit material for resynthesis." The author goes on to say "we have seen nothing impossible in such a cycle." It is true that nothing is impossible on paper, but no biochemical process is known to proceed in this manner. The hypotheses of Wrinch, of Delbrück, and of Jansenn, relating to the construction of protein molecules, have had a far greater vogue among geneticists than among protein chemists. There is no evidence for the following statements: "necessary to allow for synthesis of large portions of the protein molecule out of carbohydrate, plus ammonium salts or urea. The model for this is the synthesis of hippuric acid"; "attachment [of phosphoric acid] to nucleic acid to form polyphosphates"; "replacing the phosphoric acid with a carbon compound"; "chromatin nucleic acid . . . . is specifically thymonucleic acid"; etc. On page 16, line 3, catalase is referred to as "a cytochrome (iron-containing) enzyme." On page 7, the following statement is made: "self-reduplicating virus substance is non-fibrous, composed of peptide units sufficiently small to be completely penetrated by the interatomic electrical and electromagnetic fields which govern crystal formation." Viruses are not "self-reduplicating" in the absence of living cells; they are not nonfibrous but fibrous, if this means asymmetric in shape; most of them are not crystalline; and the phrase on the penetration by fields, etc., has no meaning. It is unfortunate to go into these details at such length, but when chemistry is given as a tool to the geneticists it should be of C. P. grade. As stated above, the review, from page 17 on, is excellent.

W. W. Pigman contributes a review on "Specificity, Classification, and Mechanism of Action of the Glycosidases." The work on this subject, chiefly by Helferich, Weidenhagen, and by Pigman himself, illustrates some of the most beautiful work done with enzymes, and the reader is urged to study this review as an illustration of what enzyme work in the hands of masters is capable of yielding. The glycosides are esters of sugars on the re-

ducing carbon, and when the possibilities of isomerism and substitution of the sugars and of the aglycone are considered, the number of available substrates becomes enormous and the number of variations to the study of enzyme-substrate equilibria correspondingly so. It is little wonder, therefore, that the study of the glycosidases has yielded so much valuable information in regard to enzymes in general.

"The Transamination Reaction" is the title of a review by R. M. Herbst. This topic is concerned with the intermolecular transfer of an amino group from an alpha amino to an alpha keto acid. This reaction is already familiar from enzymatic reactions, chiefly in muscle, but the emphasis in this review is laid on model experiments between amino and keto acids *in vitro* and the organic chemical mechanisms involved in the reactions. The model reactions differ in the following respects from the enzymatic reactions: (1) Carbon dioxide is always released by the former. (2) The model reactions are independent of the optical configuration of the reactants. And (3) the enzymatic reactions take place only if one member of the transaminating pair is a dicarboxylic amino or keto acid. Both kinds of reaction are similar in that they involve a coupled, intermolecular oxidation-reduction process. Herbst has skilfully and clearly presented the mechanisms involved. His own very extensive model experiments are valuable in revealing how the study of pure organic reactions can shed light on complex enzymatic processes. Very few similar examples exist in the chemical literature. In the midst of this splendid exposition it is regrettable that Herbst should have dabbled, though only for a moment, it is true, in the cancer field, for his brief essay into the application of transamination enzymatic studies to this field completely misses the point. It is still further surprising that the only work of value on this aspect, namely that of P. P. Cohen, should have been completely omitted from the oncological discussion, although the references to the papers of this author in *Cancer Research* are cited in the bibliography of the review.

A very extensive review on "Tyrosinase" is written by J. M. Nelson and C. R. Dawson. This enzyme occurs principally in the vegetable kingdom, where it acts by oxidizing aerobically monohydric and *o*-dihydric phenols respectively to *o*-dihydric phenols and to *o*-quinones. The authors state that "its presence in mammals has not been so easy to demonstrate." It is a pity that in so masterful and comprehensive a review the paper by Hogeboom and Adams in the *Journal of Biological Chemistry* should have been overlooked, for in this paper the presence in mouse melanomas of high tyrosinase activity has been reported. [Still higher concentrations have recently been observed in human melanomas—J. P. G.] But this is a very small flaw in an excellent review, based upon many years of research by the group at Columbia led by the senior author. The view is stated that "tyrosinase is one copper protein entity or complex possessing two enzymatic activities." This position is somewhat unorthodox to the classical enzymologist, and although it would appear that the data are susceptible to alternative interpretations, it must be admitted that the authors are very persuasive.

R. D. Hotchkiss has written a timely review on "Gramicidin, Tyrocidine, and Tyrothricin." Gramicidin and tyrocidine are products of aerobic spore-forming bacteria, have been purified as *d*-amino acid-containing polypeptides, and act as inhibitory agents on the growth of other types of bacteria. These substances have been overshadowed of late by the miracles of penicillin, and it is good to have the subject of antibiotics reviewed without mention of this substance. Hotchkiss has written a well-integrated review on the properties and biological applications of these bacterial products, which this particular reviewer, a complete ignoramus in the field, found very illuminating.

Of closest interest to the readers of *Cancer Research* is the review by V. R. Potter on "Biological Energy Transformations and the Cancer Problem." The review begins with a survey of the problems facing the investigator in the field of tumor metabolism, and the author quite rightly lays emphasis on the need for a study of the individual enzymatic systems concerned with phosphorylation, glycolysis, and respiration. The high aerobic and anaerobic over-all glycolysis of tumors may now be considered as established. Potter then goes on to develop a viewpoint of the normal mechanisms of energy transfer, and takes the position that the "adenosine triphosphate system is a common denominator by which all of the reactions of glycolysis and respiration can be made to pool their respective energies in one compound. This correlation alone makes the ATP system of paramount interest as a possible connecting link between function and the energy-yielding reactions." (p. 219.) He marshals many arguments in support of this viewpoint, which may, of course, be correct; but in some of these there is a sense of strain, as in the argument on page 220 that since calcium is related to a number of cellular processes and since calcium serves as an activator or coenzyme for ATP-ase, it follows that ATP is related to nearly everything in the cell (Fig. 3, page 223). The central position of ATP in *muscle* metabolism has long been recognized. Whether it is possible to generalize from muscle physiology to other kinds of tissues is an open question. The author states that "absence of control is the outstanding feature of cancer metabolism," that "growth impulse in cancer tissue comes from within the cells themselves," and that "growth . . . . control can be effected by means of a self-imposed deficiency of a growth factor." These statements are obviously hypothetical, but the author goes on to suggest that the breakdown products of ATP may serve as the key substances in this control. All this leads to the theory of cancer presented by him in the pages of this journal last year, which involves the presence of an unknown "cancer virus" and an "enzyme X," both of which may be nucleoproteins by rather specious reasoning. When Potter goes on to discuss his own methods of enzymatic assay of individual systems and to compile the results obtained thereby, he is on sure ground, and the reader is urged to study these pages as illustrating the finest experimental technic available in this field. This reviewer is furthermore in complete agreement with the conclusions drawn by Potter from these data, in particular that dealing with the close relation in metabolism

between tumors themselves and between tumors and homologous embryonic tissues. Whether one agrees with the speculative portions of this review or not, the entire essay is a major contribution for which Potter has put the field much in his debt.

A brief review by H. Jensen and L. E. Tenenbaum on "The Influence of Hormones on Enzymatic Reactions" is presented. It has long been recognized that the direct *in vitro* effects of various hormones on enzymatic reactions are very slight, if not negligible, while the *in vivo* effects may be entirely indirect. The authors assemble the inconclusive literature and completely disarm any criticism of their efforts by admitting in their "Conclusion" that nothing is known of the subject and that further investigations are necessary.

The last review in the volume, by W. R. Brode, on "The Absorption Spectra of Vitamines, Hormones, and Enzymes," is too short to be of value as a work of reference and contains some curious statements, which reveal the author to be more at home in the field of spectroscopy than of biochemistry. In particular the references to the proteins, such as the "resin" content of insulin, and to the nucleic acids, are rather questionable, and the description of cytochrome oxidase as a mixture of cytochromes *a, b,* and *c* is grossly inaccurate. Amino acid oxidase is not just the alloxazine-adenine nucleotide. The very interesting work on the effect of pH on shifting the absorption maximum of tyrosine, which has had important protein and histochemical applications, has been omitted. In justice to the author, this field is entirely too large to be covered adequately in a review of 42 pages and by a single investigator specializing in the physical methods.

JESSE P. GREENSTEIN

DAS GESHWULSTPROBLEM IN CHEMIE UND PHYSIOLOGIE. [The Tumor Problem in Chemistry and Physiology.] Edited by K. Hinsberg. Dresden and Leipzig: Th. Steinkopff, 1942, 434 pp. Reviewed in Schweiz. med. Wchnschr., 72:1427, 1942.

This book reviews recent work on inorganic and organic compounds in tumors, the reticuloendothelial system, the hormones, and the enzymes. The last two subjects in particular are extensively discussed.

All will agree with Hinsberg that no branch of biochemistry is so full of discordant results and explanations as the biochemistry of the neoplasms. But perhaps it is just because of this that such a book is of value. It brings scattered observations together, and it is to be hoped that their ready availability will encourage further research.

WM. H. WOGLOM